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Patrons saisonniers de transformation du carbone et efficacité métabolique des communautés bactériennes du golfe d'Amundsen, Arctique canadien

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Sommaire

Les réchauffements climatiques associés aux activités anthropiques ont soumis les écosystèmes arctiques à des changements rapides qui menacent leur stabilité à court terme. La diminution dramatique de la banquise arctique est une des conséquences les plus concrètes de ce réchauffement. Dans ce contexte, comprendre et prédire comment les systèmes arctiques évolueront est crucial, surtout en considérant comment les flux de carbone (C) de ces écosystèmes - soit des puits nets, soit des sources nettes de CO_2 pour l'atmosphère - pourraient avoir des répercussions importantes sur le climat.

Le but de cette thèse est de dresser un portrait saisonnier de l'activité bactérienne afin de déterminer l'importance de sa contribution aux flux de carbone en Arctique. Plus spécifiquement, nous caractérisons pour la première fois la respiration et le recours à la photohétérotrophie chez les microorganismes du golfe d'Amundsen. Ces deux composantes du cycle du carbone demeurent peu décrites et souvent omises des modèles actuels, malgré leur rôle déterminant dans les flux de C non seulement de l'Arctique, mais des milieux marins en général.

Dans un premier temps, nous caractérisons la respiration des communautés microbiennes (RC) des glaces de mer. La connaissance des taux de respiration est essentielle à l'estimation des flux de C, mais encore limitée pour les milieux polaires. En effet, les études précédentes dans le golfe d'Amundsen n'ont pas mesuré la RC. Par la mesure de la respiration dans les glaces, nos résultats montrent des taux élevés de respiration dans la glace, de 2 à 3 fois supérieurs à la colonne d'eau, et une production bactérienne jusqu'à 25 fois plus importante. Ces résultats démontrent que la respiration microbienne peut consommer une proportion significative de la production primaire (PP) des glaces et pourrait jouer un rôle important dans les flux biogéniques de CO_2 entre les glaces de mer et l'atmosphère (Nguyen et Maranger, 2011).

Dans un second temps, nous mesurons la respiration des communautés microbiennes pélagiques du golfe d'Amundsen pendant une période de 8 mois consécutif, incluant le couvert de glace hivernal. En mesurant directement la consommation d'O_2, nous montrons une RC importante, mesurable tout au long de l'année et dépassant largement les apports en C de la production primaire. Globalement, la forte consommation de C par les communautés

microbiennes suggère une forte dépendance sur recyclage interne de la PP locale. Ces observations ont des conséquences importantes sur notre compréhension du potentiel de séquestration de CO_2 par les eaux de l'Océan Arctique (Nguyen *et al.* 2012).

Dans un dernier temps, nous déterminons la dynamique saisonnière de présence (ADN) et d'expression (ARN) du gène de la protéorhodopsine (PR), impliqué dans la photohétérotrophie chez les communautés bactérienne. Le gène de la PR, en conjonction avec le chromophore rétinal, permet à certaines bactéries de capturer l'énergie lumineuse à des fins énergétiques ou sensorielles. Cet apport supplémentaire d'énergie pourrait contribuer à la survie et prolifération des communautés qui possèdent la protéorhodopsine. Bien que détectée dans plusieurs océans, notre étude est une des rares à dresser un portrait saisonnier de la distribution et de l'expression du gène en milieu marin. Nous montrons que le gène de la PR est présent toute l'année et distribué dans des communautés diversifiées. Étonnamment, l'expression du gène se poursuit en hiver, en absence de lumière, suggérant soit qu'elle ne dépend pas de la lumière, ou que des sources de photons très localisées justifie l'expression du gène à des fins sensorielles et de détection (Nguyen *et al*., soumis au journal ISME).

Cette thèse contribue à la compréhension du cycle du C en Arctique et innove par la caractérisation de la respiration et de l'efficacité de croissance des communautés microbiennes pélagiques et des glaces de mer. De plus, nous montrons pour la première fois une expression soutenue de la protéorhodopsine en Arctique, qui pourrait moduler la consommation de C par la respiration et justifier son inclusion éventuelle dans les modélisations du cycle du C. Dans le contexte des changements climatiques, il est clair que l'importance de l'activité bactérienne a été sous-estimée et aura un impact important dans le bilan de C de l'Arctique.

Mots-clés : océan, Arctique, glaces de mer, biogéochimie, cycle du carbone, patrons saisonniers, production bactérienne, respiration, efficacité de croissance, diversité fonctionnelle, photohétérotrophie, protéorhodopsine, expression

Abstract

Arctic ecosystems are undergoing rapid changes, primarily due to unprecedented climatic warming as a function of anthropogenic activities, which threaten their short-term stability. One of the most dramatic impacts has been the loss and change in annual sea ice. Understanding and predicting how these systems will evolve is crucial, especially if considering how carbon (C) fluxes from these ecosystems – either net sinks or net CO_2 sources for the atmosphere – could have important repercussions on global climate.

The objective of this thesis is to establish a seasonal portrait of bacterial activity to characterize its contribution to Arctic carbon fluxes. Specifically, we quantify for the first time microbial respiration in sea-ice and the water column and explore the use of photoheterotrophy by microorganism over an annual cycle in the Amundsen Gulf of the Arctic Ocean. These components of carbon cycling remain poorly understood and infrequently directly measured. As a consequence they are either extrapolated or omitted from models, despite their significant role in C dynamics not only in the Arctic, but also in marine systems in general.

First, we characterise respiration in sea-ice microbial communities (CR). An understanding of respiration rates is essential for accurate estimation of C fluxes, but the role of respiration in sea ice is poorly understood. This work represents the first comprehensive evaluation of respiration in polar sea ice to date. Using novel O_2 consumption measurements in sea-ice, we found high respiration rates in sea-ice, 2 to 3 times higher than in the water column and bacterial production rates up to 25 times higher. These results show that microbial respiration can consume a significant portion of sea ice primary production (PP) and play a key role in biogenic CO_2 fluxes between sea-ice and the atmosphere (Nguyen and Maranger, 2011).

Second, we measure respiration of pelagic microbial communities of Amundsen Gulf over an eight-month period, including under the winter ice-cover. By measuring directly O_2 consumption, we show high CR, measurable over the whole year and greatly surpassing C inputs from PP. Globally, high C consumption by microbial communities supports a high reliance on internal recycling of local PP. These observations have important consequences on our understanding of the CO_2 sequestering potential of the Arctic Ocean (Nguyen *et al.*, 2012)

Finally, we describe the seasonal patterns in presence (DNA) and expression (RNA) of the proteorhodospin (PR) gene, involved in bacterial photoheterotrophy. The PR gene, combined with the retinal chromophore, allows bacteria to capture energy from light towards energetic or sensory purposes. This additional energy source could contribute to the survival and proliferation of bacterial communities expressing the gene in the highly variable polar environment. Although PR has been found in many oceans, this study represents a unique time-series that follows the seasonal distribution and expression of the gene in a natural marine system. We show that the PR gene was present over the whole study period and widely distributed in diverse bacterial communities. Surprisingly, we observed continued PR expression over winter, in the absence of sunlight. This suggests either that the PR's expression does not depend on light or, that other very localized photon sources could justify PR expression for detection and sensory functions (Nguyen *et al.*, submitted to the ISME journal).

This thesis contributes to the understanding of Arctic carbon cycling and includes several novel elements such as the characterization of respiration and bacteria growth efficiency in both pelagic and sea-ice habitats. The use of an alternative C pathway by bacteria in the Polar ocean was also explored for the first-time in a time-series. The observed sustained expression of the PR gene in the Arctic could modulate C consumption by respiration and justify its inclusion in future models of C cycling. In a context of climate change, it is clear that bacterial activity has been underestimated and how this will change in a warmer Arctic will have a significant impact in the ecosystem's overall C budget.

Keywords: ocean, Arctic, sea-ice, biogeochemistry, carbon cycling, seasonal patterns, bacterial production, respiration, growth efficiency, functional diversity, photoheterotrophy, proteorhodopsin, expression

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a positive control. Nanopure water was used as a negative control. Conditions were kept constant between all reactions throughout the amplification procedure. We assume that within each primer pair, all other things being equal, the differences in signal intensity can be reasonably attributed to differences in initial target DNA and cDNA.

Figure S1 Map of the Amundsen Gulf region and sampled stations. Dots represent the Winter sites, while triangles represent the Spring sites. Gray symbols are sites that were chosen for cloning and sequencing.

Figure S2 Phylogenetic tree based on DNA sequences of early winter and late winter sites, generated with maximum likelihood simulations. Bootstrap values higher than 50% are shown on branches. The color of branches indicates affiliation to bacterial genera, the color strip refers to the associated lettered primer sets, and the leaf color to the period of collection (both, early or late winter). The number of affiliated sequences is presented as bar charts. Reference sequences of marine proteorhodopsin genes (white leaves) were found in GenBank. Corresponding NCBI GIs and taxonomical details are as follows in alphabetical order: Alphaproteobacterium HOT2C01 [GI:37913013 – Alphaproteobacteria.]; *Cand. Pelagibacter ubique* HTCC1002 [GI:91762240 – Alphaproteobacteria; SAR11 cluster; Candidatus Pelagibacter.]; *Dokdonia donghaensis* MED134 [GI:86130673 – Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Dokdonia.]; *Dokdonia sp.* PRO95 [GI:223452819 – Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Dokdonia.]; Flavobacteria bacterium BAL38 [GI:126663919 – Flavobacteriia, Flavobacteriales.]; Gammaproteobacterium EBAC20E09 [GI:45644626 – Gammaproteobacteria; SAR86 cluster.]; Gammaproteobacterium EBAC31A08 [GI:9971913 – Gammaproteobacteria.]; Gammaproteobacterium eBACHOT4E07 [GI:47779380 – Gammaproteobacteria; SAR86 cluster.]; Gammaproteobacterium HTCC2143 [GI:119476620 – Gammaproteobacteria; OMG group; BD1-7 clade.]; Gammaproteobacterium HTCC2207 [GI:141534101 – Gammaproteobacteria; OMG group; SAR92 clade.]; *Photobacterium angustum* S14 [GI:90580458 – Gammaproteobacteria; Vibrionales; Vibrionaceae; Photobacterium.]; *Photobacterium sp.* SKA34 [GI:89074634 – Gammaproteobacteria; Vibrionales; Vibrionaceae; Photobacterium.]; *Polaribacter irgensii* 23-P [GI:88802358 – Flavobacteriia, Flavobacteriales, Flavobacteriaceae; Polaribacter.]; *Polaribacter sp.* MED152 [GI:85819768 – Flavobacteriia, Flavobacteriales, Flavobacteriaceae; Polaribacter.]; *Psychroflexus Torquis* ATCC 700755 [GI:91216393 – Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Psychroflexus.]. *Halobacterium salinarum* (strain Shark) [GI:461612 – Halobacteria; Halobacteriales; Halobacteriaceae; Halobacterium.] was used as the outgroup. All clone sequences used to build this tree are deposited in GenBank under accession numbers KJ937475-KJ937662.

Figure S3 Number and distribution of PR OTUs between the samples sequenced in early and late winter based on GenBank affiliation of representative sequences. The Venn diagram shows the distinct and shared distribution of OTUs between dates,

while the pie charts show the number of OTUs and clones associated to each bacterial class.

Chapitre 5

Listes des abréviations

(Les caractères italiques indiquent les termes anglais.)

AB : abondance bactérienne

ATP : adénosine triphosphate

BA : *bacterial abundance*

BGE : *bacterial growth efficiency*

BLAST : *Basic local alignement search tool*

BP : *bacterial production*

BR : *bacterial respiration*

C : carbone/*carbon*

cDNA : *complementary DNA*

CFL : *Circumpolar flaw lead system study*

Chl *a* : chlorophylle a

COD : carbone organique dissous

COP : carbone organique particulaire

CR : *community respiration*

DAPI : 4',6'-diamidino-2-phénylindole

DGGE : *Denaturing gradient gel electrophoresis*

DIN : *dissolved inorganic nitrogen*

DNA *: deoxyribonucleic acid*

DOC : *dissolved organic carbon*

DIC : *dissolved inorganic carbon*

DOM : *dissolved organic matter*

ECB : efficacité de croissance bactérienne

ETS : *electron transport system*

GPP : *gross primary production*

HMW : *high molecular weight*

IWI : *ice-water interface*

LMW : *low molecular weight*

MO : matière organique

MOD : matière organique dissoute

N : azote/*nitrogen*

NCP : *net community production*

NID: azote inorganique dissous

OM : *organic matter*

OTU : *Operational taxonomic unit*

PAR : *photosynthetically active radiation*

PB : production bactérienne

PCR : po*lymerase chain reaction*

POC : *particulate organic carbon*

PP : production primaire / *primary production*

PR : protéorhodopsine / *proteorhodopsin*

R : respiration

RB : respiration bactérienne

RC : respiration des communautés

RNA *: ribonucleic acid*

rRNA *: ribosomal RNA*

TCA : acide trichloroacétique

UPGMA : *Unweighted pair group method with arithmetic mean*

À Josie-Anne et Raphaëlle

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Avant-Propos

La recherche en milieu polaire a fait des pas de géants depuis l'obtention par l'Arctique du statut de baromètre des changements climatiques. Devant la complexité des écosystèmes naturels, nous nous devions de définir une niche où la contribution de notre laboratoire serait la plus bénéfique pour l'avancement des connaissances sur la dynamique des milieux marins arctiques. Rapidement, nous avons constaté l'absence de mesure précise de la respiration microbienne dans ces milieux et, plus particulièrement, l'absence de données sur une plage temporelle de plus de quelques semaines, voire quelques jours.

Confiant de notre capacité à mesurer de très faibles taux d'activité, nous avons décidé d'orienter nos recherches en ce sens, soit la mesure de l'activité microbienne en intégrant une caractérisation des processus cataboliques associées à la respiration cellulaire. C'est avec cette idée en tête que nous sommes montés à bord du NGCC Amundsen pour près d'un an de travaux ininterrompus. Passant de la glaciale nuit arctique jusqu'au chaud – plutôt tiède - soleil de minuit, nos équipes se sont relayées à chaque 6 semaines tout au long de cette période pour amasser un jeu de donnée sans précédent sur l'écologie microbienne de l'Océan Arctique. C'est ainsi, grâce à une approche multidisciplinaire et collaborative, que nous sommes parvenus à rassembler les éléments ayant mené au chapitre 4, où par des approches moléculaires, nous tentons de lier la structure des communautés à leur fonction écosystémique.

1. Introduction

1.1. Le métabolisme microbien : une histoire de carbone

1.1.1. Microorganismes et flux d'énergie en milieux marins

Les microorganismes sont partout. À chaque seconde, ils transforment des millions de tonnes de la matière qui nous entoure et ce faisant, ils façonnent notre environnement. Abondants, ils se comptent par millions dans chaque centimètre cube d'eau ou de sol. Pour subsister, comme tous les êtres vivants, les microorganismes doivent avoir accès à une source d'énergie pour alimenter leur machinerie métabolique. À son expression la plus simple, cette énergie prendra la forme d'un flux de protons créé parallèlement à l'oxydo-réduction de la matière organique durant la respiration cellulaire. Ces processus mèneront à la génération d'adénosine tri-phosphate (ATP), la monnaie d'échange de l'énergie cellulaire. Au niveau de la cellule, cette suite de réactions est généralement réalisée durant la glycolyse, le cycle de Krebs, la chaîne de transport d'électron et la phosphorylation oxydative.

Tout débute donc avec la matière organique. Celle-ci provient en grande partie de la photosynthèse, qui réduit le carbone inorganique (c.-à-d. le CO_2) en composés organiques simples. Cette réaction, par le biais des complexes photosynthétiques et de divers pigments tels

Photo : Arrivée sur un site d'échantillonnage et déploiement des instruments et équipes, Golfe d'Amundsen, Avril 2008

que la chlorophylle, permettra de stocker l'énergie des photons solaires en énergie chimique prenant la forme, entre-autres, de glucides. Ceux-ci pourront être utilisés ultérieurement pour la biosynthèse ou le maintien de l'activité cellulaire. Cette création de matière organique, par le

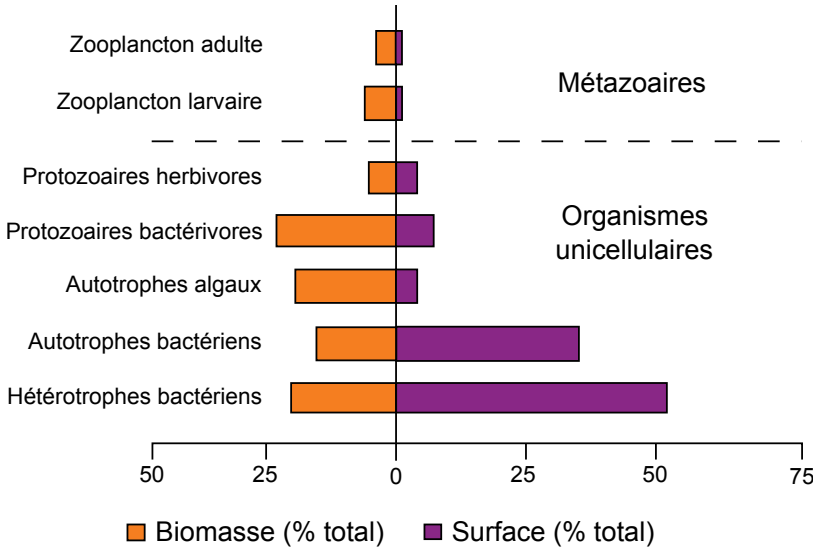


Figure 1. Distribution de la biomasse et estimation des surfaces biologiques associées, pour chacun des grands groupes trophiques planctoniques en milieu marin. Les valeurs sont exprimées en proportion (%) de la biomasse planctonique totale. Les groupes sous la ligne pointillée sont considérés comme étant des microbes. Modifié de Pomeroy et al. 2007.

biais des microorganismes photosynthétiques, est essentielle à la survie des réseaux trophiques marins. En effet, les producteurs primaires des océans, le phytoplancton, réalisent la majorité de cette tâche fondamentale. En raison de leur capacité à créer la matière organique à partir de substrat inorganique, on qualifie ces organismes d'autotrophes. L'autotrophie fait référence à leur capacité à se nourrir eux-mêmes (du grec *trophê* et *autos*) ou, d'un point de vue biogéochimique, à subvenir à leur propre besoin en carbone à partir de formes inorganiques. Au fil de l'évolution, cette production de matière organique a fait des milieux marins de vastes incubateurs pour les microorganismes, et de façon plus générale, le berceau des premiers habitants de notre planète.

Ultimement, la majorité de la production primaire sera consommée par les organismes hétérotrophes, qui dépendent de la disponibilité de cette matière organique. Les microorganismes constituent la majorité de ces consommateurs, autant en nombre qu'en biomasse. De plus, les parois cellulaires de ces organismes invisibles représentent la majorité des surfaces biologiques

de l'océan, une composante très importantes pour les réactions biogéochimiques (Figure 1.1, Pomeroy *et al.*, 2007). L'écologie microbienne des milieux marins est très dynamique et implique des communautés diversifiées, capable d'utiliser la moindre ressource disponible grâce à la grande plasticité de leurs voies métaboliques. Parmi ce monde de microbes, le métabolisme des bactéries marines mérite une attention toute particulière et sera au centre de cette thèse.

1.1.2. Le cycle du carbone (C) marin

Les océans occupent 71 % de la surface terrestre et réalisent environ la moitié de la production primaire (PP) globale (Field *et al.,* 1998). En effet, en utilisant la matière organique dissoute (MOD), les bactéries ont accès au bassin de 650-700 • 10^{15} g C de MOD que contiennent les océans (Hansell et Carlson 2001). Leur capacité à utiliser cette matière sous ses formes les plus diverses a fait d'eux les moteurs des grands cycles biogéochimiques planétaires (Falkowski *et al.,* 2008). Par exemple, l'incorporation de C organique en biomasse lors de la production bactérienne (PB) permet le transfert de MOD vers les maillons trophiques supérieurs. Ce processus est bien décrit par le concept de la boucle microbienne (Azam *et al.,* 1983), un concept central à la compréhension du cycle du C marin.

Conceptuellement, la boucle microbienne a introduit la notion du recyclage de la matière organique par l'intermédiaire des bactéries. Déjà en 1974, Lawrence Pomeroy énonçait les premières bases du concept en décrivant l'importance potentielle des microbes (dans le cas présent : la fraction des organismes de taille inférieure à 1µm) dont on commençait à mesurer l'importante contribution au métabolisme marin (Pomeroy 1974). Avec ce «nouveau paradigme», les microorganismes, incluant les bactéries, passaient à l'avant-scène de la consommation de carbone organique et des flux d'énergie en milieu marin. Toutefois, on ne pouvait alors déterminer clairement l'existence d'un lien avec le réseau trophique classique. Les microbes représentaient-ils un puits ou une source de carbone organique pour les maillons trophiques supérieurs?

Les premières réponses ne viendront qu'une dizaine d'années plus tard, avec l'avancement des méthodes de dénombrements direct par épifluorescence (Hobbie *et al.,* 1977, Porter et Feig 1980) qui permettront de mieux quantifier le rôle des communautés microbiennes. Ainsi, en 1983, Farook Azam parlera pour la première fois d'une «boucle microbienne» permettant la réintroduction du carbone organique dissous, perdu tout au long du réseau

trophique classique, par le broutage des bactéries par les ciliés et flagellés (Azam *et al.,* 1983). Les bactéries deviennent donc non seulement de grandes consommatrices de C mais également

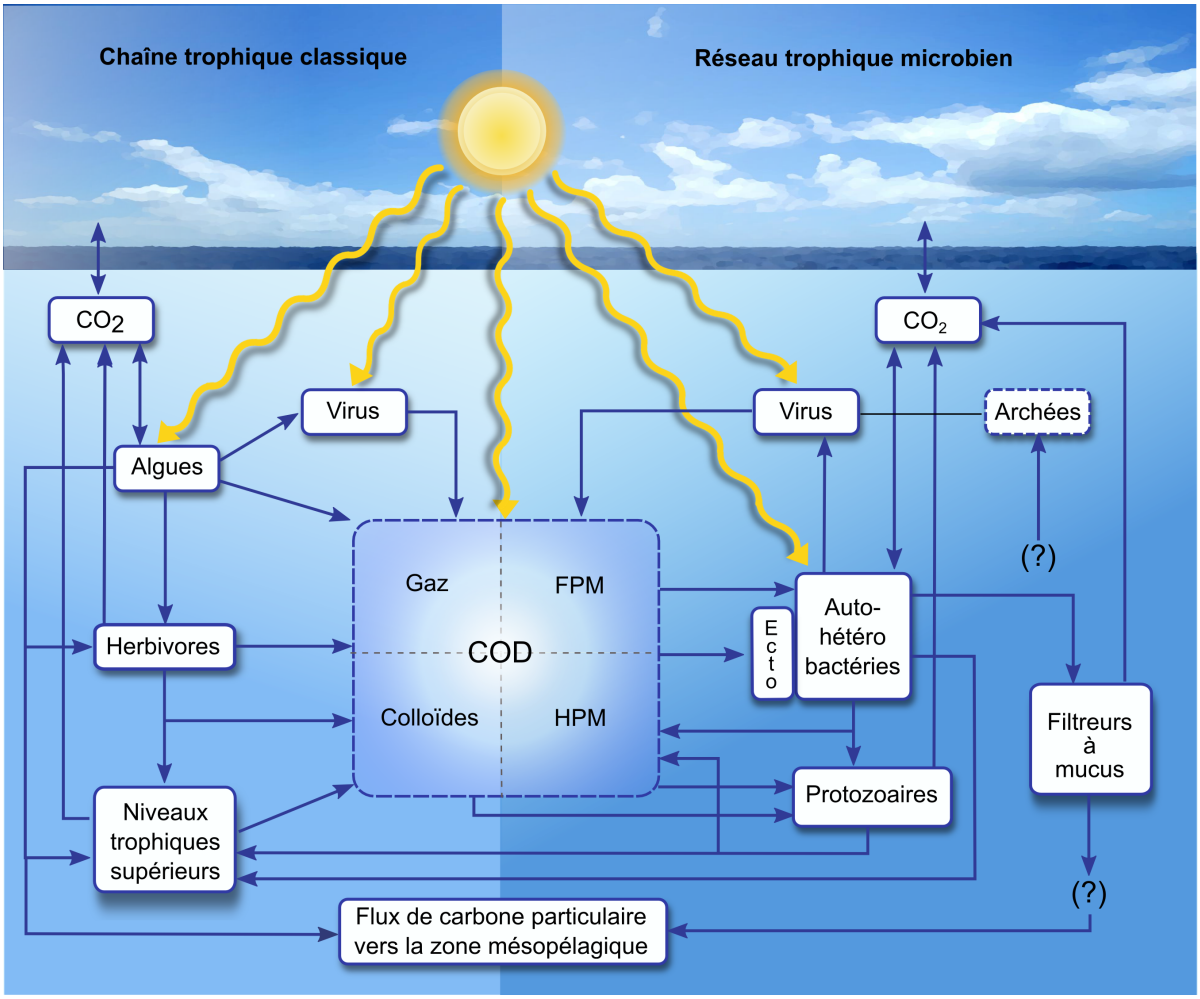


Figure 2. Représentation schématique du réseau trophique marin montrant à gauche la conception classique des flux de carbone et d'énergie passant par les eucaryotes photosynthétiques, puis les herbivores et ainsi de suite aux maillons supérieurs. Représenté à droite est le réseau trophique microbien, qui utilise l'énergie stockée dans le carbone détritique, non-vivant, pour produire de la biomasse microbienne, qui peut être réintroduite dans le réseau trophique classique. Des ecto-enzymes associées aux cellules permettent aux bactéries d'utiliser le COD de haut poids moléculaire (HPM) en plus des formes plus biodisponibles de faible poids moléculaire (LPM). (Adapté de Delong et Karl, 2005)

des sources de C pour les brouteurs. De cette façon, la production des bactéries ré-alimentent la base du réseau trophique en énergie, qui autrement serait perdue par le système. Plus récemment, avec le développement rapide des méthodes -omiques, des ressources importantes ont été investies pour tenter de lier la diversité microbienne aux fonctions biogéochimiques des systèmes (DeLong 2009). Bien que notre compréhension de leur rôle demeure rudimentaire, les avancées

dans la caractérisation du carbone organique dissous (COD), des archées et de la prédation bactérienne par les bactériophages ont mené à leur inclusion dans les conceptualisations plus récentes des réseaux trophiques microbiens (DeLong et Karl 2005, Figure 1.2).

1.1.3. Le métabolisme bactérien

Depuis, les recherches ont majoritairement utilisé les mesures de PB pour quantifier la contribution bactérienne aux réseaux trophiques aquatiques. Toutefois, pour incorporer la consommation bactérienne de C à un bilan de C, la PB n'est pas le processus le plus informatif. En effet, comme la PB implique un transfert du C organique dissous (COD) vers le pool organique particulaire (COP) sous forme de biomasse bactérienne, elle n'est ni une entrée, ni une sortie de C organique. L'efficacité de ce transfert est estimée par l'efficacité de croissance bactérienne (ECB) qui permet d'estimer les pertes en C organiques du système par l'effet combiné de la production et la respiration bactérienne (RB) (Jahnke et Craven 1995). La caractérisation de ces pertes lors du transfert de C organique vers le C inorganique par la RB, nécessite une mesure directe de la respiration et est critique pour la détermination du bilan de C d'un écosystème. Ainsi, il sera soit net autotrophe, produisant suffisamment de C localement (C autochtone) pour alimenter le réseau trophique ou net hétérotrophe dans le cas où le système nécessite des apports externes en C (C allochtone) pour subvenir aux besoins des organismes hétérotrophes. Pace *et al.,* (2004) ont montré que les apports allochtones pouvaient être importants en milieu aquatique et fournir l'énergie nécessaire non seulement au métabolisme bactérien, mais à l'ensemble du réseau trophique.

La RB est le catabolisme par lequel les bactéries oxydent des sources de carbone pour alimenter leurs besoins métaboliques en énergie. Physiologiquement, que ce soit chez les prokaryotes ou les eukarytotes, ces réactions sont associées à l'activité du cycle de Krebs et du système de transport d'électron (ETS) qui permettront l'oxydation phosphorylative. Ce dernier assure l'oxydation terminale des substrats produits en amont par l'oxydoréduction de l'accepteur final d'électron soit, en aérobie, l'oxygène (White 2000). Ainsi, la respiration, en consommant la MOD et l'oxygène et en libérant du CO_2, peut représenter un flux majeur de C pour les océans (Williams et del Giorgio 2005). Les bactéries sont responsables de la majorité de la respiration des écosystèmes aquatiques (Williams et del Giorgio 2005) et représentent souvent de 50 à >90 % de la respiration totale (Rivkin et Legendre 2001, Robinson *et al.,* 1999, Sherr *et al.,*

1999). Néanmoins, il semble que ce ne soit pas toujours le cas, certaines études démontrant une contribution estivale plus modérée des bactéries dans l'ouest de l'océan Arctique (Kirchman *et al.,* 2009a).

Pour relier le C incorporé par la PB aux pertes de C organique associées à la RB, les chercheurs auront recours à l'efficacité de croissance bactérienne (ECB= PB / (PB+RB)). Malgré sa propension à varier grandement dans le temps et l'espace (del Giorgio et Cole 1998), et à défaut de mesure directe de la RB, on a longtemps assumé des ECB élevées de 40 à 60 %, qui minimisaient l'importance de la RB dans le cycle du C aquatique. Avec l'intensification des mesures de la RB, del Giorgio et al. (1997) ont constaté que pour la plupart des milieux oligotrophes l'ECB était plutôt inférieure à 20 %, modifiant radicalement notre conception de l'importance et du rôle des bactéries dans le cycle du C. De récentes mesures effectuées en eaux froides et oligotrophes montrent des ECB encore plus faibles, de 6.9 % en moyenne (Kirchman *et al.,* 2009a), suggérant une autre sous-estimation de l'importance de la RB dans le cycle du C. D'autres études récentes (Kritzberg *et al.,* 2010, Ortega-Retuerta *et al.,* 2012), confirment cette tendance à de faibles ECB (<10%) en Arctique durant l'été. Néanmoins, la RB demeure une variable peu mesurée directement en raison de limites méthodologiques et logistiques. Les rares mesures enregistrées tendent à être biaisées vers les mesures en surface, en régions tempérées et durant la saison chaude (del Giorgio et Williams, 2005), qui présentent des taux plus élevés et plus aisément mesurables que les taux des eaux profondes et froides. Ceci devient problématique lorsque l'on considère que la majorité du biome océanique est caractérisé par des eaux froides et profondes (< 4 °C et > 1000 m). Ainsi, même si les taux de respiration en eaux froides sont faibles, leur contribution pourrait être majeure vu l'important volume considéré.

1.2. L'activité microbienne en Arctique

1.2.1. Particularités de l'Arctique

L'océan Arctique, par sa relative petite taille et les nombreuses rivières qui le ceinturent, est fortement influencé par les apports externes en matière organique qu'il reçoit. Une hausse de température associée aux changements climatiques pourrait avoir des répercussions importantes sur la respiration. En effet, les modèles prédisent non seulement les plus importantes hausses de température en Arctique (GIEC), mais également une augmentation de la PP (Arrigo *et al.,* 2008)

et des apports allochtones en C (Peterson *et al.,* 2002, Peterson *et al.,* 2006), pouvant tous stimuler l'activité microbienne et la respiration. Sans données plus étendues sur la respiration, il est difficile de prédire comment ces changements influenceront ce flux de C en Arctique. À l'échelle globale, la RB semble pousser les milieux vers l'hétérotrophie, surtout dans le cas des milieux oligotrophes (Cole 1999, del Giorgio *et al.,* 1997). Quelques études en milieux polaires ont montré que la PP pouvait répondre aux besoins en C des hétérotrophes, sans toutefois avoir mesuré directement la respiration (Kottmeier *et al.,* 1987, Moran *et al.,* 2001, Rich *et al.,* 1997). Dans une baie du golfe d'Amundsen, Garneau et al. (2008) ont montré une activité bactérienne mesurable tout au long de l'hiver. Ils concluaient que des apports en C issus de la production passée devaient alimenter les prokaryotes durant la saison froide.

Historiquement, on a assumé que les eaux froides de l'Arctique limitaient la respiration, et le faisaient saisonnièrement pencher vers l'autotrophie. Cette prémisse n'a jamais vraiment été remise en question dans les budgets de C arctiques, alors que l'on tend à montrer que les bactéries sont capables d'activité métabolique à très faibles températures, même sous le point de congélation (Junge *et al.,* 2004). Pour cette raison, la respiration est peu considérée dans les budgets de C arctiques et calculée indirectement à partir de mesure de PP ou bactérienne, en assumant une efficacité de croissance donnée (Williams et del Giorgio 2005). On assume que ces modèles reflètent bien la respiration, alors qu'elle n'est pas réellement caractérisée. L'utilisation de tels procédés permet une estimation grossière de la respiration, qui demeure utile pour établir un budget de C préliminaire et approximatif. Toutefois, une mesure directe permettrait de confirmer ou d'infirmer la validité de ces modèles et, par le fait même, d'améliorer notre compréhension du cycle du C en Arctique.

1.2.2. Les glaces de mer

Les glaces de mer sont une composante importante des écosystèmes polaires. En occupant environ 7 % de la surface du globe, elles représentent un écosystème d'une étendue non-négligeable (Comiso 2003, Horner *et al.,* 1992). Avec la diminution estimée du couvert de glace de près de 10 % par décennies, cet environnement risque aussi d'être le plus touché par les changements climatiques (Comiso 2006). La glace est un milieu dynamique, hétérogène et soumis au régime des saisons qui en affecte son étendue, sa biologie et ses propriétés physiques et chimiques, comme la densité, la salinité, la température et la luminosité (Eicken 2003). Les

organismes qui s'y trouvent sont majoritairement concentrés dans les canaux saumâtres qui se créent à la formation de la glace et qui sont caractérisés par une salinité plus haute qui empêche leur gel. Lors de la formation de la glace, algues, bactéries et autres protistes se trouvent emprisonnés dans ces canaux ou entre les cristaux de glace et se développent plus ou moins indépendamment de la colonne d'eau, selon la porosité et densité de la glace (Horner *et al.,* 1992, Lizotte 2003).

Ces glaces, reconnues pour la PP qui s'y effectue (Gosselin *et al.,* 1997, Horner et Schrader 1982), constituent un milieu également propice pour les bactéries (Junge *et al.,* 2004). La luminosité, déterminée par l'épaisseur de glace et le type et l'épaisseur de la couverture de neige (Mundy *et al.,* 2005), affecte directement les autotrophes en limitant leur croissance et indirectement les hétérotrophes en influençant les concentrations en composés dissous issus de la PP (Riedel *et al.,* 2006, Smith *et al.,* 1997). En effet, il existe un lien positif entre ces composés et l'abondance des communautés bactériennes (Bunch et Harland 1990, Kahler *et al.,* 1997, Riedel *et al.,* 2007). La glace pourrait également représenter une source importante de COD pour le milieu et fournir, lors de sa fonte, un substrat labile pour les communautés de la colonne d'eau. L'étendue minimale des glaces de mer arctique a chuté drastiquement au courant des dernières années, et se situait nettement sous les moyennes historiques (1979-2013), avec une valeur minimale record en 2012 (National Sea-Ice Data Center, NSIDC). Dans ce contexte, il importe d'obtenir rapidement une mesure de référence de l'activité microbienne dans les glaces de mer si nous souhaitons mieux comprendre et identifier les conséquences des perturbations climatiques.

1.2.3. Facteurs contrôlant l'activité bactérienne en eaux froides

Physiologiquement, qu'il s'agisse de l'eau ou de la glace, les organismes croissant à faibles températures, les psychrophiles, possèdent des optimums de croissance inférieurs à ceux de leurs homologues tempérés (Grossi *et al.,* 1984). À faible température, on observe des optimums de croissance de 4 à 8 °C pour les bactéries et inférieurs à 15 °C pour les autres hétérotrophes (Kottmeier et Sullivan 1988, Lizotte 2003). Ces organismes doivent parvenir à maintenir leur machinerie protéique malgré les limites imposées par le froid et trouver des stratégies pour maintenir l'affinité des enzymes aux substrats, la fluidité de leurs membranes et lipides et posséder des enzymes à faible énergie d'activation (Feller et Gerday 2003, Gounot et Russell 1999). Grâce à ces adaptations, ils parviennent à demeurer actifs à basse température et

continuent à remplir leur fonction écologique, indépendamment de la température. La disponibilité des substrats et des nutriments est également à considérer, puisqu'elle peut influencer le métabolisme bactérien (Goldman *et al.,* 1987, Kirchman *et al.,* 2005).

Quelques études ont tenté de déterminer comment la température influençait la production et la croissance bactérienne par l'estimation de Q_{10} (Apple *et al.,* 2006, Kirchman *et al.,* 2005, Kritzberg *et al.,* 2010, Yager et Deming 1999). Le Q_{10} nous informe sur la hausse anticipée du métabolisme avec une hausse de 10 °C. Leurs résultats suggèrent une influence variable de la température selon la gamme de température considérée. En général, on considère qu'un même changement de température de 2 °C n'aura pas les mêmes conséquences s'il se fait de -2 à 0 °C ou de 6 à 8 °C. La hausse du taux de croissance de -2 à 4 °C serait non-linéaire, pour ensuite tendre à la linéarité après 4 °C (Kirchman *et al.,* 2009b). Dans le cas de la RB, Apple et al. (2006) ont observé une réponse plus forte de celle-ci aux changements de température que la PB dans un estuaire tempéré, une observation intéressante dans le contexte des réchauffements climatiques.

La disponibilité et la qualité des substrats modulent également l'effet de la température et tendent à faire diminuer son influence, particulièrement pour les communautés adaptées au froid (Autio 1998, Pomeroy et Wiebe 2001, Rasmussen *et al.,* 2003). Toutefois, certains auteurs croient plutôt que les substrats agissent indépendamment de la température (Kirchman *et al.,* 2005, Lopez-Urrutia et Moran 2007). En présence de communautés autotrophes, les bactéries peuvent s'approvisionner à partir de C relativement jeune, considéré plus labile, facilement assimilable et riche en nutriments. Ce C, issu de la PP, est associé à une hausse de l'ECB par son effet positif sur la PB (del Giorgio et Davis 2003). Un autre réservoir de C est celui du COD résiduel, plus âgé, provenant de la PP passée. Peu disponible biologiquement, il caractérise les zones profondes et oligotrophiques des océans (del Giorgio et Duarte 2002). Ce C de poids moléculaire élevé, pauvre en nutriments et difficile à assimiler est considéré comme récalcitrant. De plus, certaines bactéries possèdent la capacité de sécréter des ecto-enzymes pour faciliter son assimilation (DeLong et Karl 2005). Ces processus enzymatiques, demandants en énergie, augmentent la respiration et sont associés à une plus faible BGE (del Giorgio et Davis 2003).

Par leur relativement faible ratio C : N, les bactéries ont des besoins importants en N. Généralement, elles préfèrent des formes organiques simples comme les acides aminés libres, mais peuvent également utiliser des formes inorganiques (NID) comme le NH_4^+ ou le NO_3^-

(Kirchman 2000). Comme les acides aminés libres sont rapidement utilisés, l'utilisation de NID peut être importante pour soutenir la croissance bactérienne, surtout en présence de matière organique à haut ratio C : N (Caraco *et al.,* 1998, Caraco et Cole 2003). Expérimentalement, un faible ratio C : N du substrat, plutôt que sa source, semble mener à une hausse de la ECB des bactéries marines (Goldman *et al.*, 1987). En utilisant des bioessais, Jorgensen et al. 1999 ont proposé qu'une plus grande incorporation d'acides aminés indiquait une plus grande disponibilité en N, expliquant ainsi les taux plus faibles de RB et la plus grande ECB dans un estuaire tempéré par rapport aux sites marins. Des études ont montré un certain rôle du P dans la hausse de la PB, mais avec un impact limité ou nul sur la RB (Smith et Prairie 2004).

1.2.4. Diversité fonctionnelle des communautés bactériennes

Mais que se passerait-il si les microbes pouvaient accéder à des sources d'énergie autres que la matière organique? Les microbes ont longtemps été considérés comme un groupe fonctionnel relativement uniforme, effectuant des processus et occupant des fonctions similaires dans les écosystèmes aquatiques. Certains font même référence à une «boîte noire» microbienne pour illustrer notre ignorance des subtiles interactions microbiennes. Par exemple, les années 1970-80 ont été marquées par la découverte de deux classes de pico-cyanobactéries photoautotrophes, synechococcus (Johnson *et al.*, 1979) et prochlorococcus (Chisholm *et al.*, 1988) dont l'existence était jusque-là insoupçonnée. Ubiquitaires, ces cyanobactéries coccoïdes allaient s'avérer des contributrices majeures à la production primaire océanique, en réalisant environ 25% de la production primaire planétaire (Kirchman 2012). Ainsi, bien que notre compréhension du lien entre la structure des communautés microbiennes et leur fonction demeure limitée, les dernières décennies ont permis des avancées importantes dans la caractérisation et la description des communautés microbiennes (DeLong 2009, Gasol *et al.,* 2008). L'utilisation du gène ribosomal 16S comme marqueur phylogénétique (Giovannoni *et al.*, 1990) a levé le voile sur la diversité microbienne. L'avènement des méthodes de séquençage à haut débit (Venter *et al.*, 2004, Edwards *et al.*, 2006) a fait progresser de façon exponentielle notre connaissance des génomes et métagénomes microbiens. Plus récemment, l'identification et le suivi de gènes impliqués dans des processus d'intérêt écologique et biogéochimique ont posé les bases du concept de la diversité fonctionnelle microbienne. La découverte dans les océans de processus hétérotrophes alternatifs utilisant l'énergie de la lumière par l'entremise de la

bactériochlorophylle (Kolber *et al.,* 2000, Kolber *et al.,* 2001) ou la protéorhodopsine (Beja *et al.,* 2001, Béjà *et al.,* 2000) – la photohétérotrophie – pourrait changer notre compréhension du rôle des microbes dans l'océan. Depuis, plusieurs études suggèrent que le gène de la protéorhodopsine serait commun en milieu marin (Finkel *et al.,* 2013, Rusch *et al.,* 2007). De même, il semble que la lithohétérotrophie (c.-à-d. l'oxydation du CO inorganique comme source d'énergie additionelle afin d''assimiler le C organique) soit une autre stratégie assez répandue (DeLong et Karl 2005, Moran et Miller 2007) mais relativement peu étudiée. Récemment, (Alonso-Saez *et al.,* 2010) ont observé une utilisation importante du C inorganique, sous forme de bicarbonate, par les bactéries arctiques, leur donnant un potentiel avantage compétitif lorsque les formes organiques sont limitantes. Bien que la respiration semble relativement constante malgré la variation des communautés bactériennes présentes (Reinthaler *et al.,* 2005), les processus alternatifs sont peu documentés et pourraient nous mener à revoir notre conception des flux d'énergie en milieu marin. Les études tendent toutefois à démontrer leur prévalence dans plusieurs milieux aquatiques, particulièrement dans le cas de la photohétérotrophie (Cottrell et Kirchman 2009a, Finkel *et al.,* 2013, Rusch *et al.,* 2007).

1.2.5. Impact potentiel des changements climatiques

Les réchauffements climatiques pourraient affecter tous les facteurs de contrôle de la RB. À défaut de connaissances suffisantes, nous ne pouvons qu'émettre des hypothèses sommaires sur les effets potentiels sur la RB sur la séquestration ou l'émission de CO_2 par les océans. Ainsi, dans le cas où les processus autotrophes sont dominants et permettent un export net de matière organique vers les profondeurs, on pourra parler de séquestration. Par contre, dans le cas où la respiration et le recyclage de la matière organique par la boucle microbienne – incluant la contribution des brouteurs et bactériophages – surpassent les processus autotrophiques, on parlera plutôt d'émission biologique nette de CO_2 par les océans. Ainsi, une hausse de la respiration, dans le cas où la PP ne hausserait pas dans les mêmes proportions, risque d'amplifier l'impact des réchauffements climatiques. Les hypothèses concernant le couplage ou le découplage du métabolisme bactérien avec la PP sont contradictoires (del Giorgio et Duarte 2002, Hoppe *et al.,* 2008, Kirchman *et al.,* 2009a). Dans le cas où la respiration serait fortement couplée avec la PP, on peut s'attendre à un certain équilibre entre séquestration et émission de CO_2 par les océans. Dans ce cas, une extrapolation de la respiration à partir de modèles basés sur la PP donnerait une

approximation valable des changements à venir. Par contre, dans le cas du découplage entre respiration et PP, la respiration pourrait augmenter de façon non linéaire alors que la PP resterait relativement stable avec un réchauffement des océans, déséquilibrant le budget de C Arctique. Dans ce cas, nous devrons être en mesure de prévoir indépendamment la variation de la respiration. Pour se faire, une meilleure compréhension non seulement des taux de respiration en eaux froides mais également des facteurs qui la régulent s'avèrent nécessaire. Ces informations permettront de développer de nouveaux budgets de C qui intègreront mieux les impacts des changements climatiques sur le cycle du C en Arctique.

1.3. Structure générale et objectifs de la thèse

Cette étude a pour objectif de dresser un portrait saisonnier de l'activité bactérienne dans l'océan Arctique. Pour ce faire, la thèse est structurée autour de cinq chapitres. Le chapitre 1 se veut un survol du cycle du C et de la boucle microbienne marine et présente les spécificités des régions arctiques. Le chapitre 2 présente l'importance du métabolisme microbien dans le milieu complexe et diversifié des glaces de mer. Le chapitre 3 vise à étendre nos mesures de respiration dans le plus grand contexte spatiotemporel de la zone pélagique, où nous caractérisons le rôle du métabolisme microbien dans les flux de C de l'écosystème. Le chapitre 4 explore la diversité fonctionnelle des bactéries marines par l'entremise du gène de la protéorhodopsine, un gène impliqué dans l'activité photohétérotrophique chez les procaryotes. Finalement, le chapitre 5 présente nos principales contributions au savoir et discute de quelques perspectives d'avenir pour l'étude de l'activité microbienne en Arctique. Dans son ensemble, cette thèse dresse un portrait saisonnier de l'étendue et du dynamisme du métabolisme bactérien dans les milieux arctiques et pose les bases d'une meilleure incorporation de la composante microbienne, particulièrement la respiration, dans les modèles biogéochimiques arctiques.

1.3.1. Objectifs spécifiques

Chapitre 2. Caractériser la respiration des communautés microbiennes des glaces de mer

Le chapitre 2 examine l'importance de l'activité bactérienne dans la glace et à l'interface glace-eau. Du mois de mars à juillet 2008, nous avons réalisé 7 expériences de mesure de la respiration dans la glace. Un suivi de l'activité bactérienne a également été réalisé dans l'eau de l'interface glace-eau, directement sous la glace. Nos résultats montrent une activité microbienne

soutenue dans la glace et une augmentation de la PB avec le retour de la saison chaude. Il semble qu'une proportion significative de la PP de glace est consommée par la RC et pourrait jouer un rôle important dans les flux biologiques de CO_2 entre la glace et l'atmosphère.

Chapitre 3. Mesurer la respiration microbienne à travers plusieurs saisons et caractériser pour la première fois ce flux déterminant pour le golfe d'Amundsen.

Le chapitre 3 a pour objectifs de 1) caractériser la consommation de C dans le golfe d'Amundsen par la production et la respiration bactérienne, 2) d'en déterminer les principaux facteurs de contrôle et 3) d'intégrer et comparer ces flux aux bilans de carbone existant pour les régions arctiques. Pour ce faire, une mesure hebdomadaire de la respiration microbienne et bactérienne fut réalisée de novembre 2007 à juillet 2008 dans les eaux du golfe d'Amundsen. Parallèlement, des échantillons furent collectés pour la mesure de la PB, l'abondance bactérienne (AB), la chlorophylle *a* et les nutriments (NO_3^-, NH_4, PO_4, Si). Au total, 51 mesures de la respiration dans la colonne d'eau furent effectuées avec succès durant le projet CFL. Nos résultats montrent une RB importante au travers des saisons se traduisant par une efficacité de croissance très faible et en moyenne de trois à cinq fois inférieure aux valeurs utilisées dans les modèles précédents. La forte consommation de C par la RC confirme l'importance du recyclage interne de la PP locale dans le golfe d'Amundsen. Nous concluons sur les répercussions importantes sur le bilan de C arctique et l'importance d'intégrer la mesure de la respiration aux variables couramment mesurées en Arctique.

Chapitre 4- Lier la structure des communautés microbiennes à leur fonction écologique par le suivi du gène de la protéorhodopsine chez les communautés procaryotiques du golfe d'Amundsen

Le chapitre 4 détermine l'évolution temporelle de la présence et de la diversité *in situ* de la PR chez les communautés procaryotiques polaires. Par le suivi de l'expression du gène, nous tentons d'élucider son impact sur l'écologie microbienne du milieu et son implication dans les fonctions énergétiques ou sensorielles du bactérium. La prévalence de la PR pourrait non seulement avoir une forte influence sur le cycle du C arctique, mais également être très dynamique en raison des cycles saisonniers très polarisés, passant d'une clarté continue stimulant une forte PP, à une nuit éternelle et une quasi-absence de PP en hiver. Une expression soutenue

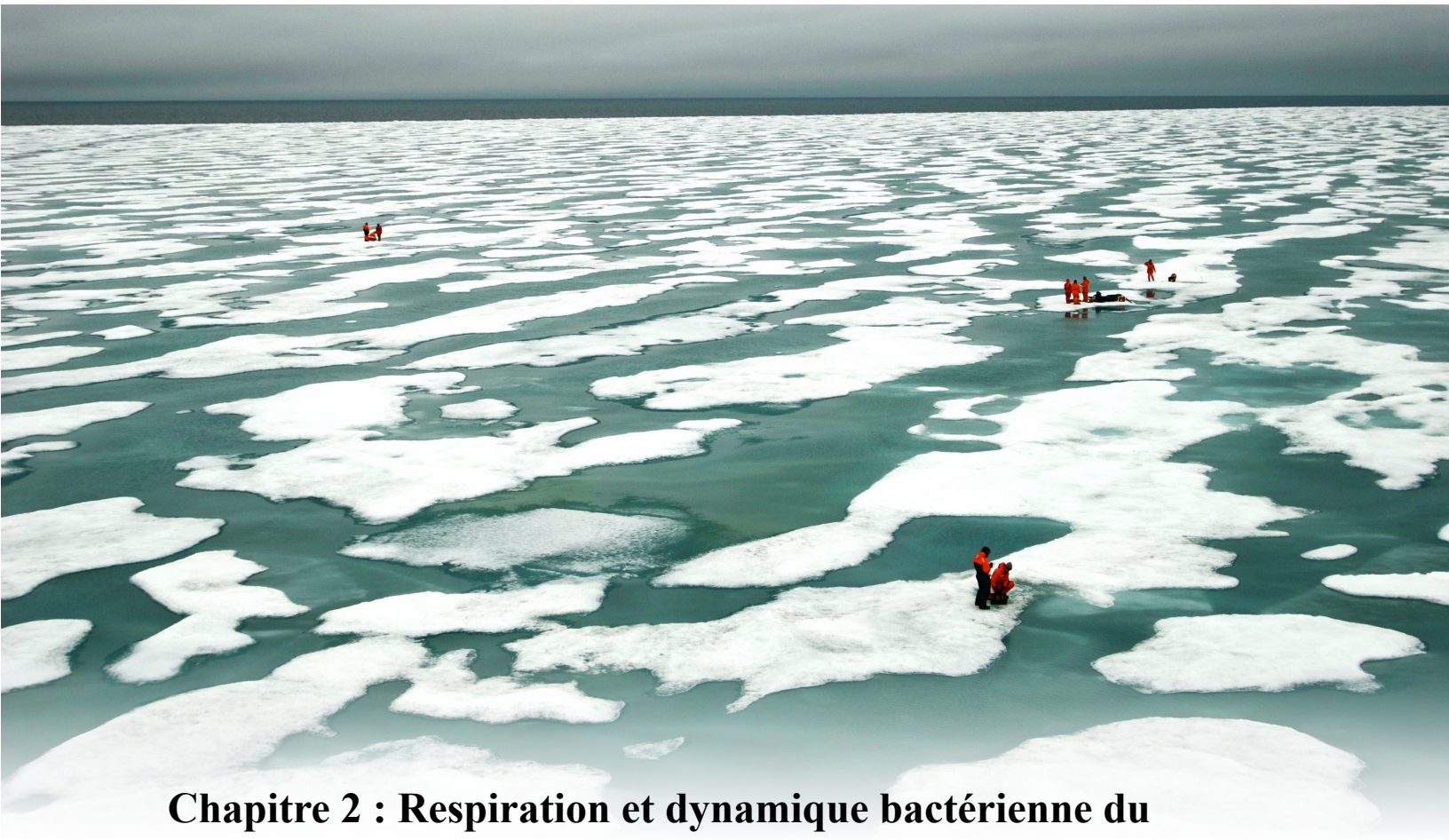
du gène pourrait nous forcer à réviser notre conception du rôle des bactéries dans les flux de C en milieu marin. Nous discutons des patrons observés et des indicateurs qui suggèrent une implication du gène dans les fonctions sensorielles plutôt qu'énergétique.

1.4. Cadre général de l'étude

1.4.1. Le projet Circumpolar Flaw Lead system study

Notre étude porte sur les eaux et les glaces de la mer de Beaufort, plus précisément celles du golfe d'Amundsen. Nos mesures ont été réalisées à bord du brise-glace NGCC Amundsen dans le cadre du projet *Circumpolar Flaw Lead Study 2007-2008* (CFL) durant l'Année Polaire Internationale (IPY-API). Cette mission multidisciplinaire et internationale s'est étendue sur plus d'une année, au cours de laquelle nous avons échantillonné la colonne d'eau de novembre 2007 à juillet 2008 et la banquise de mars à juillet 2008. Cette période marque le passage d'un couvert de glace complet à l'eau libre, de la nuit polaire au soleil de minuit et est reconnue comme étant très dynamique pour les processus biologiques et écologiques.

L'objectif de la mission était de caractériser la physique, chimie et la biologie de la colonne d'eau et des glaces dans les ouvertures (*flaw*) se formant dans la banquise sous l'effet des vents, du courant et de la température. Ainsi, CFL se voulait un suivi des observations effectuées durant la mission CASES 2004 (Canadian Arctic Shelf Exchange Study) et cherchait à comprendre le rôle écologique de ces ouvertures, qui risquent de devenir de plus en plus fréquentes avec les changements climatiques. Les membres de l'équipe 7 Flux de Carbone, avaient le mandat de suivre l'évolution des processus bactériens et de la diversité microbienne tout au long du projet CFL. La nature collaborative du projet CFL nous a donné un accès privilégié au riche jeu de données amassées par plus de 200 scientifiques composant les 10 équipes du projet.



Chapitre 2 : Respiration et dynamique bactérienne du carbone dans les glaces de mer Arctique

Photo : Échantillonnage des glaces de mer, Golfe d'Amundsen, Mai 2008

Respiration and bacterial carbon dynamics in Arctic sea ice

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Abstract

Bacterial carbon demand, an important component of ecosystem dynamics in polar waters and sea ice, is a function of both bacterial production (BP) and respiration (BR). BP has been found to be generally higher in sea ice than underlying waters, but rates of BR and bacterial growth efficiency (BGE), are poorly characterized in sea ice. Using melted ice core incubations, community respiration (CR), BP and bacterial abundance (BA) were studied in sea ice and at the ice-water interface (IWI) in the Western Canadian Arctic during the spring and summer 2008. CR was converted to BR empirically. BP increased over the season and was on average 22 times higher in sea ice as compared to the IWI. Rates in ice samples were highly variable ranging from 0.2 to 18.3 μg C L^{-1} d^{-1}. BR was also higher in ice and on average ~10 times higher than BP, but was less variable ranging from 2.39 to 22.5 μg C L^{-1} d^{-1}. Given the high variability in BP and the relatively more stable rates of BR, BP was the main driver of estimated BGE (r^2=0.97, $p<0.0001$). We conclude that microbial respiration can consume a significant proportion of primary production in sea ice and may play an important role in biogenic CO_2 fluxes between the sea ice and atmosphere.

Keywords: Arctic Ocean, sea ice, respiration, bacterial production, BGE, C cycling

2.1 Introduction

Estimating rates of major biogeochemical cycles is a great challenge in the Arctic environment and for sea ice in particular. Annual polar sea ice is a dynamic habitat characterized by steep physical and chemical gradients that permit the establishment of a diverse micro-fauna critical to sustaining polar food webs (Lizotte 2001, Michel *et al.,* 1996). The expulsion of salts and nutrients from the ice through brine channels, create nutrient rich conditions at the growing ice front (Bunch and Harland 1990) supporting dense autotrophic and heterotrophic communities (Thomas and Dieckmann 2010). Autotrophic production in sea ice during the spring has been the topic of several studies because of the high rates of ice algal primary production and impressive biomass accumulation (Arrigo *et al.,* 2010, Gosselin *et al.,* 1986, Riedel *et al.,* 2006). Indeed, ice algal production can contribute up to one third of the total primary production (PP) in the Arctic (Legendre *et al.,* 1992) and therefore plays an important functional role in ecosystem dynamics of polar seas.

The increase in solar radiation during spring rapidly induces blooms in the bottom ice resulting in the release of dissolved organic compounds by ice algae, producing critical substrates that support heterotrophic microbial communities (Riedel *et al.,* 2006, Smith *et al.,* 1997). Bacterial abundance (BA) and bacterial production (BP) in sea ice typically follow increasing algal production and biomass during the spring bloom (Bunch and Harland 1990, Kottmeier *et al.,* 1987, Kuparinen *et al.,* 2007, Smith *et al.,* 1989). The ratio of BP to PP ranges from 10 to 30% in the literature (Cole *et al.,* 1988, Ducklow 2000). However, Garneau et al. (2008) observed a higher ratio in the Franklin Bay of Amundsen Gulf, with BP accounting for 37% of PP. Although heterotrophic bacterial biomass and rates of production can be very high in sea ice, rates of bacterial respiration remain largely unknown. In other aquatic systems, bacterial respiration almost always exceeds BP (del Giorgio *et al.,* 1997), making respiration the most important fate of bacterial carbon and a significant contributor to the whole system metabolism.

Respiration quantifies carbon going from the organic to the inorganic pool, for a net output of carbon, while BP is a transfer of carbon from the dissolved organic (DOC) to the particulate organic carbon (POC) pool, and is therefore neither an input nor output of carbon to the ecosystem (Jahnke and Craven 1995) Respiration is essential to accurate carbon budgetting and represents an important carbon sink. Respiration estimates, be it of bacteria or the whole

microbial community, are relatively rare as compared to BP, not only for the Arctic and for sea ice but for all aquatic ecosystems, although there is a growing effort to fill this important gap (Williams and del Giorgio 2005). Where estimates are available for polar and subpolar regions, bacterial respiration accounts for the bulk of community respiration, ranging from 50 to >90% (Sherr and Sherr 1996; Robinson et al. 1999; Rivkin and Legendre 2001). However, a recent estimate for the Chukchi Sea suggests a more variable contribution from bacteria (Kirchman *et al.,* 2009a).

To avoid often complex and time consuming measurements of respiration, most studies elucidating the role of bacteria in ecosystem carbon dynamics have calculated bacterial respiration (BR) from empirically derived bacterial growth efficiency (BGE). BGE relates the total amount of C incorporated into biomass relative to the total bacterial carbon demand (BCD), which is the sum of BP+BR (BGE=BP/BCD). Recent studies have clearly shown much lower BGEs than the conversion factors commonly used to estimate BR especially in the polar ocean (del Giorgio *et al.,* 1997, Kirchman *et al.,* 2009a) implying that bacterial community carbon requirements are considerably higher then previously assumed.

Considering the rapid decrease of multi-year Arctic sea ice and potentially greater extent of biologically productive seasonal ice combined with reduction in snow cover (Arrigo *et al.,* 2008), there is a pressing need to understand microbial carbon cycling in sea ice, specifically bacterial respiration, as it may be an important sink for organic carbon. The main objective of this study was to measure bacterial production, community respiration and bacterial growth efficiency in sea ice samples from the Amundsen Gulf during the spring bloom and compare these estimates with the underlying water. These novel data provide new insight on C cycling dynamics in sea ice.

2.2 Material and methods

2.2.1 Study site

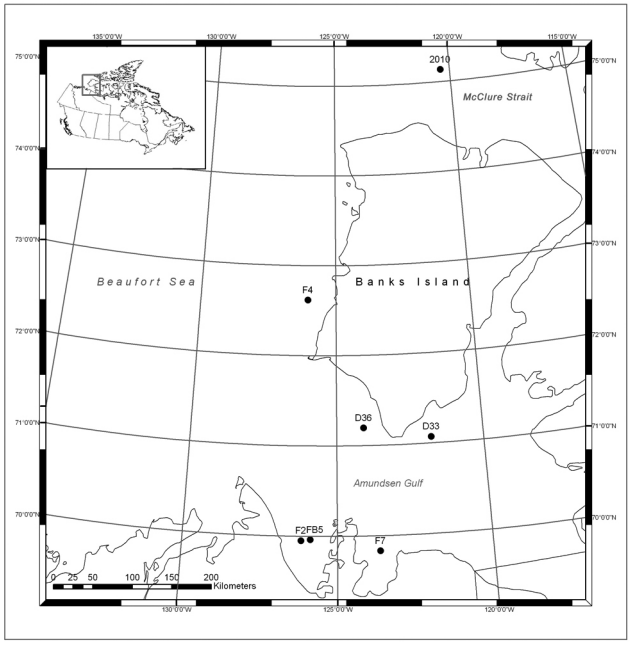

Fig. 1 Study area showing location of stations sampled for sea ice and underlying seawater. Inset shows global map of Canada for spatial reference.

Sampling was carried out between March and July 2008 in the Amundsen Gulf of the Arctic Ocean (Figure 1) onboard the *CCGS Amundsen* as part of the International Polar Year–Circumpolar Flaw Lead system study (IPY–CFL). A total of seven sites (Table 1) were sampled for ice cores and underlying water primarily from sites located in the Amundsen Gulf, with one site in the Beaufort Sea adjacent to multiyear ice and one site in McClure Strait (Figure 1). This period marks the transition from the Arctic winter to the Arctic summer and is very dynamic biologically. Only one site (D33) permitted the sampling of both high and low snow cover.

Spring 2008 was characterized by a lower than average ice extent, with a predominance of first-year ice and an earlier onset than usual of melting in the Amundsen Gulf (Barber *et al.,* 2010). By the end of June, the Amundsen Gulf was free of ice, and the ship headed toward McClure Strait, north of Banks Island, to find seasonal sea ice that could be sampled (Figure 1, station 2010). Station F4 located in the Beaufort Sea was the only site neighbouring multiyear fast-ice. The rapid loss of ice, combined with the need to sample open water, resulted in sampling ice over a greater spatial extent. This greater extent can be expected to introduce more variability

in the data, but the dynamic conditions did not allow us to return to the same location over the season.

2.2.2 Sample collection and processing

Ice cores were extracted using a 9 cm diameter ice corer (Mark II, Kovacs Enterprise). The bottom 4.5 cm of ice was collected in sterile whirl-pak bags (Nasco) until sample processing. This section was chosen because of it is association with high microbial autotrophic and heterotrophic activity, as our main objective was to monitor biologically mediated C fluxes. At each site, 2 L of water at the ice-water interface (IWI, directly under the ice, < 1 m depth) was collected using either a Kemmerer bottle or a submersible pump and stored in opaque acid washed bottles. For logistical reasons, surface water from site F2 was collected at a depth of 10 m. All samples were kept in an isothermic container to avoid sudden temperature changes and limit exposure to direct sunlight.

Samples were melted to measure rates of processes. Since microbial communities are mostly located in brine channels, ice samples were melted in 900 mL of filtered seawater (FSW, 0.2 μm) to avoid osmotic shock, achieving a final FSW:ice melt ratio in the range of 3:1 to 4:1 (Garrison and Buck 1986, Maranger *et al.,* 1994). Melting was carried out in a cold room onboard, in the dark, at 2 - 4 °C to avoid sudden temperature changes and allow equilibration to incubation temperature. Subsamples were taken for bacterial abundance and bacterial production measurements. For respiration measurements, 500 mL Erlenmeyer flasks were carefully filled with the remaining sample water to avoid bubble formation and sealed gas tight using a thick silicon stopper and water resistant tape. The use 0.2 μm FSW may not have removed the smallest of bacterial cells and allowed passage of viruses, however dilution with melted water of this size fraction is commonly used for rate estimates in sea ice. Given that all sea ice samples were treated equally we believe the rates remain comparable among samples and variables, and serve as potential rates when compared with the water column.

Logistically, sea ice samples are melted to permit the measurement of various biological rate estimates but melting obviously changes the *in situ* sea ice matrix and other environmental conditions. Samples are diluted to reduce the shock of melting on microbial communities. We acknowledge that both steps, commonly taken in the study of sea ice, will influence our measures with respect to *in situ* conditions. Furthermore dilution in FSW may have decreased grazer

concentrations while increasing virus numbers relative to BA. Throughout this paper, when we refer to rates measured in sea ice, we imply rates measured in melted sea ice samples and corrected for dilution in FSW. The resulting rates should be viewed as estimates of potential rates that can help constrain bacterial metabolism in sea ice.

Respiration rates were determined on whole communities, as samples were not prefiltered to isolate bacterial communities; BR was estimated using an empirical model (Robinson 2008) as described below. This decision was taken based on previous studies showing that polar bacterial communities in both sea ice (Junge *et al.,* 2004) and water column (Garneau *et al.,* 2009), although very different (Bowman *et al.,* 1997), can have a strong affinity for particles. Pre-filtration was avoided as we anticipated low rates and wished to conserve the in situ community assemblage as much as possible. Indeed, a greater effect from predator omission is expected when using size fractionation (Robinson and Williams 2005) to selectively remove particle-attached and larger organisms, rendering extrapolation to *in situ* conditions more tenuous. Algal respiration also occurred during CR incubations. However, as shown by (Ikeya *et al.,* 2000), respiration of sea ice diatoms decreases rapidly at low irradiance, suggesting a limited effect on global CR rates.

2.2.3 Bacterial abundance, cell biovolume and bacterial biomass

Bacterial abundance (BA, cell mL^{-1}) was determined using epifluorescence microscopy of DAPI (4'6'-Diamidino-2-phenylindole dihydrochloride) stained cells (Porter and Feig 1980). Briefly, 20 mL water samples were fixed with 1 mL formaldehyde (37%) and stored in the dark at 2 - 4 °C until processing. Samples (10-20 mL) were filtered onto 0.2 µm polycarbonate black filters (25 mm, Millipore) to which 50 µL of DAPI (0.5 mg mL^{-1}) stain was added. The stain was left on the filter for 5 minutes before completing the filtration. Towers were rinsed with 5 mL of ultrapure water to minimize bacterial adhesion to tower walls and distribute cells evenly on the filter. Filters were mounted on slides and stored at -20°C until photographed using a digital camera (Canon Canada) mounted on a Leica epifluorescence microscope. Counts were done by image analysis using the ImageJ software (Abramoff *et al.,* 2004). For each slide, seven fields or more were photographed and analyzed, and a minimum of 200 cells was counted for each slide. Cell biovolume (V, μm^3) was calculated using these images with the equation $V = 4\pi r^3/3 + (l - 2r)\pi r^2$ assuming a cylindrical shape with two hemispherical caps (Smith and Prairie 2004).

Spherical cells occur when $l = r$. Cells with a volume greater than 0.344 μm^3 were not considered bacterial (Gasol *et al.,* 1995, Straza *et al.,* 2009). To estimate bacterial biomass, mean C content per cell (CC, fg $cell^{-1}$) was calculated using a conversion factor of 148 fg C μm^{-3} (Kirchman *et al.,* 2009a). Bacterial biomass (BB, µg C mL^{-1}) was calculated using $BB = CC \bullet BA / 10^9$.

2.2.4 Bacterial production

Bacterial production was measured using the ^{3}H-leucine incorporation method (Smith and Azam 1992). Water samples (1.2 mL) were dispensed, in triplicate, into clean 2 mL microcentrifuge tubes pre-loaded with 50 µL ^{3}H-leucine (115.4 Ci $mmol^{-1}$, Amersham) to produce a final leucine concentration of 10 nM (Garneau *et al.,* 2008). Samples were incubated in the dark in an ice-filled isothermic container (-1.8 to 0°C) for approximately 4 h. Leucine incorporated into cell protein was collected after precipitation by trichloroacetic acid (TCA) and centrifugation. Tubes were filled with 1.25 mL liquid scintillation cocktail (ScintiVerse, Fisher Scientific) and radioactivity was measured using a Tri-Carb 2900 TR Packard Liquid Scintillation Analyzer. Rates of leucine incorporation were corrected for radioactivity adsorption using TCA killed controls and converted to bacterial C production (BP) using two conversion factors 0.9 and 1.5 kg C per mol^{-1} ^{3}H-leucine (Garneau et al. 2008). Rates in sea ice were corrected for initial dilution in FSW. Cell specific BP (BP_{sp}) was calculated as BP/BA.

2.2.5 Community Respiration and potential bacterial growth efficiency

Community respiration (CR) was calculated by measuring the change in dissolved O_2 concentrations with fiber optic optodes (Fibox, PreSens, Germany) adapted from methods described by Kragh et al. (2008) and Marchand et al. (2009). Briefly, 500 mL Erlenmeyer flasks were carefully filled with melted ice and IWI samples to avoid bubbles, then sealed air tight with a thick rubber stopper and waterproof tape. An O_2 sensitive sensor spot (sensor type PSt3, 5mm diameter) was fixed to the internal wall of the flask where O_2 concentrations were measured by linking a light emitting (600-660 nm) optical fiber to the sensor spot (from the outside of the bottle). The sensor emits more or less luminescence depending on O_2 concentration in the sample. Measurements were not affected by salinity or pH and were corrected for sample temperature. Unlike other methods, the use of optodes has the advantage of leaving the

incubation unperturbed with almost real time O_2 measurements, is relatively space efficient and does not produce toxic waste.

Incubations were carried out in duplicate for 5 to 10 days in a temperature controlled cold room and bottles were kept in the dark submerged under water in isothermic containers to minimize temperature variations and prevent gas exchange. O_2 concentrations were measured at 24 to 48 h intervals. Temperature was kept at 2 - 4°C during all incubations. For each station, a 500 mL Erlenmeyer flask containing only ultrapure water was used as control. In all incubations, O_2 concentrations in the control were either stable or increased slightly, suggesting possible underestimation of CR rates. To adjust rates to reflect potential rates at *in-situ* temperature, we used Q10 values of 3.8 (Apple *et al.,* 2006) and 13.8 (Yager and Deming 1999) to account for variability in the temperature response of bacteria. An average Q10 value of 8.8 was used for statistical analyses and bacterial growth efficiency (BGE) estimates. Values were converted to C respired ($\mu g\ C\ L^{-1}\ h^{-1}$) assuming a respiratory quotient of 0.8 (Robinson *et al.,* 1999, Williams and del Giorgio 2005) and rates were corrected for initial dilution in FSW. Linear rates were observed throughout the incubation period, suggesting limited impact of containment on measured rates (Robinson 2008). For quality control, any incubation with an r^2 below 0.75 was to be rejected. The mean r^2 during incubation periods was 0.94 and no incubations or replicates were rejected (r^2 range: 0.76 to 0.99).

Bacterial respiration (BR) was estimated using an empirically derived equation reported by Robinson (2008), where $BR=0.45CR^{0.93}$. This equation allows for variation in the proportion of CR attributed to bacteria, and results in a lower relative proportion of BR at high CR rates. Using this equation, the mean percentage of CR attributable to BR in this study was 44%, ranging from 40 to 49%. Kirchman et al. (2009a) observed lower percentages in the Chukchi Sea with an average of 25%. However that estimate excludes those values reported in Kirchman et al. (2009a) where BR and CR were co-equal. When those 100% values are included the average BR increases to 44% of CR. Although we acknowledge that in some cases BR may be overestimated (or underestimated), the present empirical conversion (Robinson 2008) is currently the most robust option. Estimated proportions are within the average range of recent reports (Robinson and Williams 2005; Robinson 2008), and conservative according to some studies (Rivkin and Legendre 2001, Robinson *et al.,* 1999). Potential cell specific BR (BR_{sp}) was calculated using $BR_{sp}=BR/BA$. As BA likely changed over the course of the sample incubations, values for BR_{sp}

need to be interpreted with caution. Potential BGE estimates were calculated as the ratio BP/(BP+BR).

Several conversion factors were used to estimate CR, BR and BP, which will impact the estimates of BGE. For this reason we have referred to BGE as a potential estimate. We were conservative in choosing values for respiratory Q10 and conversion of community respiration to BR, but true values were likely more variable than those we assigned. Finally, the fact that BP and BA were only measured at the beginning of CR incubations may have introduced artefacts in the data. We found evidence to support the validity of our methodology in Robinson (2008), where multiple studies showed a limited effect of longer incubations on CR, whereas long incubations for BP resulted in nonlinear increases in BP rates. Roland and Cole (1999) observed no significant effect of initial BA on BP, BR and BGE measurements. Others found that increased BA during incubations was not accompanied by increases in CR (Robinson and Williams 2005). These studies support the contention that while methods used in the present study could be improved, the rates and relationships described therein are reasonable estimates of sea ice microbial dynamics.

2.2.6 Statistical analysis

Type 1 linear regressions and non-linear relationships were calculated using SigmaPlot 10 (Systat Software Inc) for Windows. One-tailed Student's t-tests for difference of means were computed with SPSS 17.0 for MacOSX (SPSS Inc). The one-tailed hypothesis was based on reports showing higher substrate availability in sea ice that could fuel bacterial metabolism. Non-parametric Spearman rank correlation tests were computed using The R Statistical package (R Core Team, 2011) . Data were log-transformed when necessary to meet normality assumptions of the test used.

2.3 Results

Average values, range and coefficient of variation (CV) of each measured variables in melted sea ice and ice-water interface (IWI) samples are presented in Table 2. Overall, sea ice showed higher bacterial activity and numbers than underlying waters. BP was highly variable over the sampling period (CV= 120% in ice and 87% in IWI; Table 2) and significantly higher in sea ice than IWI by a factor of 22 on average ($t=-1.892$, $df=10$, $p\leq0.05$). CR was also

significantly higher in sea ice as compared to IWI (t=-1,97 *df*=12, p≤0.05), but by a much smaller factor of 3.2. Estimates of BR were on average 40 to 49% of CR and 2.8 times higher in sea ice. BP increased over time in both sea ice and IWI (Fig. 2A) but rates were consistently lower in IWI (Fig. 2B). Interestingly, sea ice BR rates showed no trend over time in sea ice (Fig. 2C), whereas BR increased slightly in IWI over the study period (Fig. 2D). Overall, CVs in both sea ice and IWI were lower for BR than BP.

Table 1 General physical characteristics and sampling dates of sites in this study.

Site	Date	Location	Water body	Latitude	Longitude	Ice type	Ice thickness	Snow thickness	Freeboard	Bottom ice temp.	Air Temp.	Chl *a*
	DD/MM/YY			dec. deg.	dec. deg.		cm	cm	cm	°C	°C	μg L^{-1}
D33	25/03/08	South of Banks Is.	Amundsen Gulf	71.0650	-121.7867	drift	144	3	10.5	-1.9	-27.3	57.1
D36	09/04/08	South of Banks Is.	Amundsen Gulf	71.1895	-124.0867	drift	71	1	6	-2.4	-13.5	237
F2	17/05/08	Franklin Bay	Amundsen Gulf	69.9467	-126.1720	landfast	171	0	13	-1.8	-1.6	1970
F4	24/05/08	West of Banks Is.	Beaufort Sea	72.6101	-126.0364	landfast	126.5	8	9	-2.2*	1.4	0.70
F7	08/06/08	Darnley Bay	Amundsen Gulf	69.8267	-123.6308	landfast	143	0	8	-1.2	1.8	1.62
FB5	15/06/08	Franklin Bay	Amundsen Gulf	69.9563	-125.8750	landfast	165	8	12	-0.6**	1.3	4.30
2010	07/07/08	North of Banks Is.	McClure Strait	75.1280	-120.3966	landfast	NA	0	NA	-0.7	6	NA

* Temperature taken at a nearby site on May 20th
** Temperature taken at a nearby site on June 18th
NA= Data not available

Table 2 Average values, ranges, standard deviation (SD) and coefficient of variation (CV) for variables measured in this study (abbreviations as defined in the text). The asterisk denotes values significantly higher ($p \leq 0.05$) in melted sea ice samples (ICE) than in the ice-water interface (IWI) using a one-tailed Student's t-test for differences of means

Variable	**ICE (N=7)**				**IWI (N=7)**				**ICE:IWI**
	Mean	Range	SD	CV %	Mean	Range	SD	CV %	Mean ratio
BP (μgC $L^{-1}d^{-1}$)									
CF=0.9	4.3*	0.15-13.7	5.1	120	0.32*	0.04-0.79	0.28	87	22
CF=1.5	7.1*	0.25-22.8	8.5	120	0.53*	0.06-1.31	0.47	87	
CR (μgC $L^{-1}d^{-1}$)[A]									
Q10=3.8	46.8*	18.3-75.5	20.2	43	25.6*	6.7-63.3	18.3	40	3.2
Q10=13.8	23.4*	9.7-37.0	10.1	43	13.9*	3.4-33.4	9.9	40	
Potential BR (μgC $L^{-1}d^{-1}$)[AB]	14.4*	6.2-22.5	5.8	40	8.4*	2.39-19.6	5.6	37	2.8
BA ($x10^5$ cells mL^{-1})	2.99	0.71-5.74	1.7	57	2.27	0.47-5.87	1.8	77	2.2
Cell volume (μm^3 $cell^{-1}$)	0.106	0.078-0.125	0.02	17	0.110	0.057-0.16	0.04	32	1.04
CC cell (fg C $cell^{-1}$)	15.7	11.5-18.6	2.6	17	16.2	8.5-23	5.2	32	
BP_{sp} (fg C $cell^{-1}$ d^{-1})	15.2*	0.70-31.8	10.5	61	4.7*	0.5-22.4	8.7	186	12
BR_{sp} (fg C $cell^{-1}$ d^{-1}) [AB]	68.3	10.7-150	45.3	85	58.5	16-186	61.9	105	2.4
Biomass (μg C L^{-1})	4.9	1.12-10.4	3.3	67	4.2	0.8-13.2	4.3	104	2.6
SGR (d^{-1})	0.90*	0.061-1.76	0.56	58	0.29*	0.049-1.36	0.52	90	11
SRR (d^{-1}) [AB]	11.3	1.34-22.4	7.8	91	9.08	1.7-26.2	9.2	76	2.6
Potential BGE (%) [AB]	24.0*	0.98-74.3	24.8	105	5.8*	1.0-11.4	4.0	118	15

[A]=Q10 corrected values

[B]=Based on the equation $BR=0.45CR^{0.93}$ (Robinson 2008)

Potential BGE tended to increase with time in ice (Fig. 2E) and in underlying waters (Fig. 2F), and were both highly variable among sampling dates as shown by their respective CVs of 105 and 118% (Table 2). High variability in BP and relatively less variability in BR resulted in the wide range of BGEs observed, from 0.98 to 74%. The absence of significant relationship between BP and BR suggests a level of uncoupling of both variables, although this result could be an artefact of the small sample size in our study. The variability in BGE followed a highly significant hyperbolic relationship with BP when combining data from both the sea ice and IWI (Fig. 3) (r^2=0.97, n=12, p<0.0001). No relationship was observed between BGE and BR.

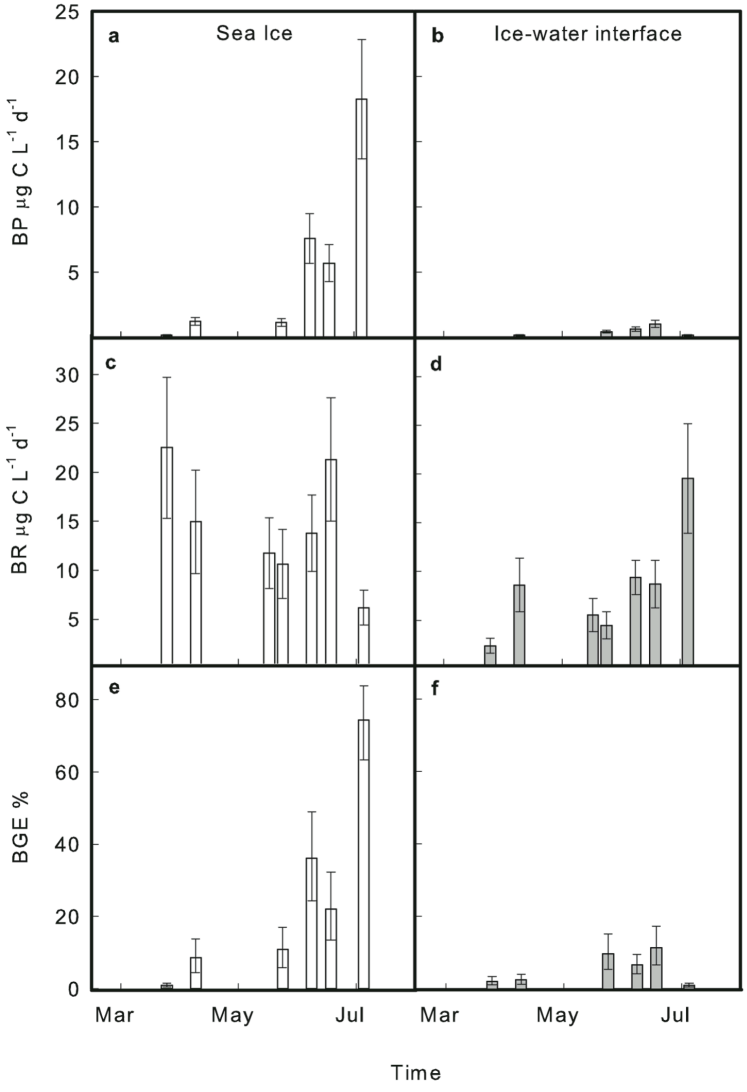

Fig. 2 Rates of bacterial production (a, b), bacterial respiration (c, d) and potential bacterial growth efficiency (e, f) over the course of spring 2008. Values for melted sea ice are displayed as white bars in a, c and e and ice-water interface as gray bars in b, d and f. Error bars are minimum and maximum estimates that originate from range of conversions used. [1]

[1] BP values are based on the mean of triplicates, while BR values are the mean of duplicates. Variations between replicates are not shown on this graph.

BA tended to increase from March to July in sea ice (Fig. 4A), and on average, was higher by a factor of 2.2 as compared to IWI (Table 2), but this difference was not statistically significant. Cell volume followed a similar increase with time in ice (Fig. 4B) but there was no clear time trend in IWI. Higher values of BA in sea ice and no significant difference in cell

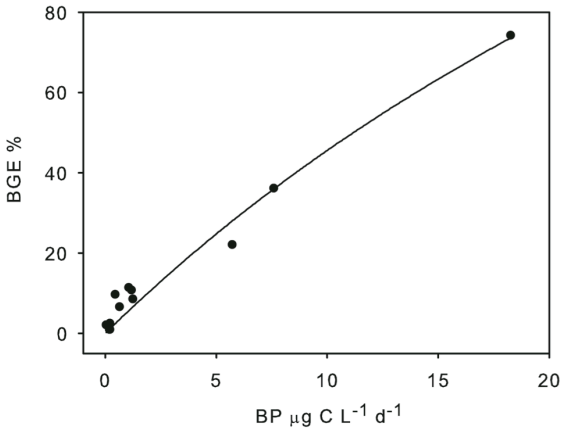

Fig. 3 Hyperbolic relationship between BGE and bacterial production for all data, melted sea ice and ice-water interface combined.

volume resulted in a higher overall biomass (Table 2). A significant positive relationship was observed between BA and BP (r^2=0.40, n=12, p=0.026), whereas no relationship was observed between BA and BR.

Cell specific rates of production and respiration remained higher in sea ice as compared to IWI, although with comparatively lower ICE:IWI ratios than community rates, 10.6 and 2.9 for

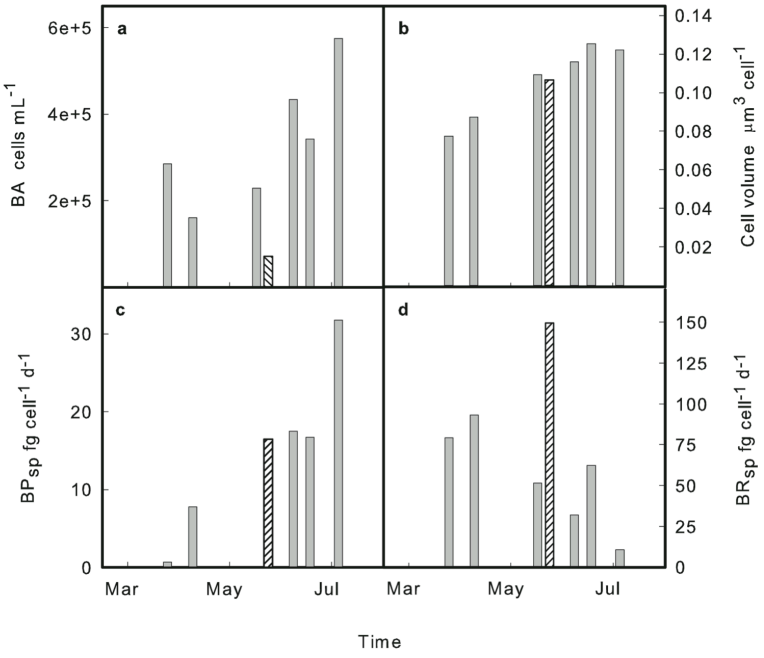

Fig. 4 Temporal trend in observed bacterial abundance (a), cell volume (b), specific bacterial production (c) and specific bacterial respiration (d). All values are for melted sea ice only. The hatched bar denotes station F4 which had the lowest BA.

BP_{sp} and BR_{sp} respectively. Over time, BP_{sp} behaved similarly to community BP (Fig. 4B), while BR_{sp} differed by showing a marked decrease during the same period (Fig. 4D).

For BGE a different pattern was observed at the cell-specific level, where patterns of cell activity in ice and IWI differed. Although our sample size was small, BR_{sp} (log) was the main driver of BGE in ice (Fig 5B, r^2=0.89, n=6, p=0.005). While no significant linear pattern was observed between BGE and $BP_{sp,}$ a non-parametric Spearman rank correlation test showed perfect fit (*rho*=1, n=6, p<0.001), indicating a non-linear relationship between these variables. IWI followed a positive linear relation between BGE and BP_{sp} (Fig 5A, r^2=0.69, n=6, p<0.05) albeit with a significantly shallower slope. There was no relationship between BR_{sp} and BGE in the IWI.

Specific growth rates (SGR, d^{-1}), as calculated from BP rates and standing biomass, increased over time, while specific respiration rates (SRR, d^{-1}), decreased during the same period (not shown). SRR was on average approximately 10 times higher then SGR (Table 2), suggesting high respiratory C demand in both ice and underlying waters.

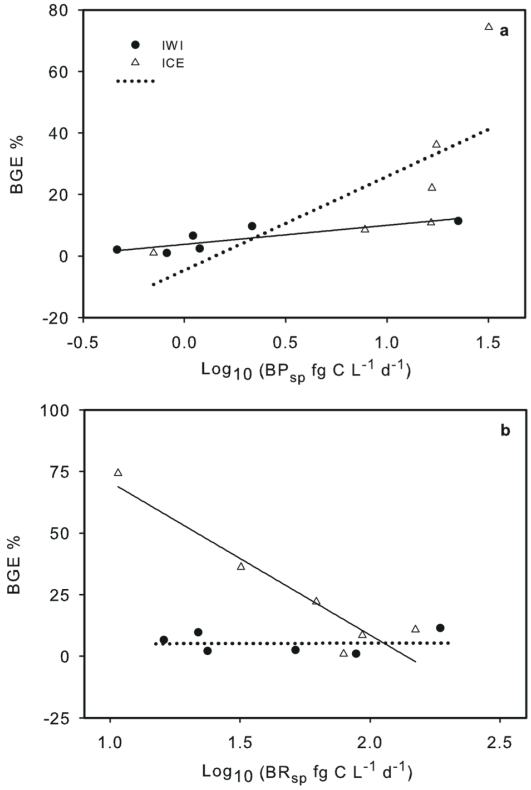

Fig. 5 Relationships between BGE and a) specific bacterial production (r^2_{IWI}= 0.69, p<0.05) and b) specific bacterial respiration ($r^2_{sea\ ice}$= 0.89, p<0.01). Open triangles represent melted sea ice values and dots ice-water interface values. Significant linear relationships are shown as full lines, non significant as dotted lines.

2.4 Discussion

This study clearly shows that microbial respiration can be a major sink of organic C in sea ice. BR rates were on average an order of magnitude higher than bacterial production in both sea ice and IWI. Although bacterial production and abundance in sea ice have been described in previous studies (Table 3), few have reported respiration rates in this critical Arctic habitat and ours is unique in attempting to characterize bacterial growth efficiency and its seasonal dynamics. To our knowledge, only one report of respiration in sea ice exists where community rates of 6.9 to 26.2 µg C L^{-1} d^{-1} (0.72 to 2.73 µmol L^{-1} d^{-1}) were observed in early April in Franklin Bay, in the Western Canadian Arctic (Rysgaard *et al.,* 2008). Our CR rates of 9.7 to 75.5 µg C L^{-1} d^{-1} (Table 2), represent a longer seasonal view of the dynamics, and widen the range at the upper end of reported values for sea ice.

2.4.1 Bacterial abundance and biomass

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Estimates of bacterial abundance (BA) and biomass in sea ice vary widely, but our estimates fell within the lower end previously reported ranges (Table 3), driven in part by the low BA (7 x 10^4 cells mL^{-1}) observed at site F4. This site was the only one located in the Beaufort Sea (Table 1) and near a multiyear ice pack formation (approximately 300 m away), but no variable we measured explain the low BA. Despite our relatively low abundances, BA was consistently higher in sea ice relative to the underlying water (even if the mean difference was not statistically different; Table 2) as previously reported (Delille 1992, Maranger *et al.,* 1994). Surprisingly, cell volume was similar in ice and underlying waters, in contrast to the general expectation of larger cells within sea ice (Delille and Rosiers 1996, Mock *et al.,* 1997). One possible explanation for this result is the use of the 0.344 um^3 limit for identifying bacterial cells, which would have excluded any very large bacteria from our counts. However, results using higher cut-offs (up to 0.7 μm^3) did not reveal any obvious difference in cell size between ice and IWI. Nevertheless, our average estimates for cell biovolume in sea ice are still 50 to almost 400% higher than recently reported estimates in the water column of the Chukchi Sea (Kirchman et al. 2009a) supporting that not only bacteria in sea ice, but also those at the ice-water interface, are larger than cells deeper in the water column.

2.4.2 Respiration, bacterial production and BGE

The range of respiration rates was surprisingly narrow, constrained to within an order of magnitude, especially when considering the 3.5 month-long temporal range of this study that encompassed a much wider range of chlorophyll values in bottom ice (Table 1). BR was on average ~ 3 times lower in the IWI as compared to sea ice, but rates tended to increase in the IWI towards the end of spring. Additional inputs of organic matter coming from the melt waters of the ice and the associated increase in temperature, could explain enhanced bacterial metabolism (Kirchman *et al.,* 2005, Pomeroy and Wiebe 2001). Overall, respiration rates measured in this study were in agreement with other reported measures of BR and/or CR in Arctic waters (Cottrell *et al.,* 2006, Kirchman *et al.,* 2009a, Kritzberg *et al.,* 2010) and in sea ice (Rysgaard *et al.,* 2008). The previously reported range for CR of 0.192 to 191 μg C L^{-1} d^{-1} suggests more variable rates in the Arctic water column, as compared to the more constrained values we observed in ice.

Cell specific rates of BR (BR_{sp}), were considerably more variable in our samples, where rates decreased over the course of the season when abundance increased. The latter should be interpreted with caution however as 1) BA likely changed over the course of respiration incubations and 2) BR was estimated using an equation by Robinson (2008).

Regardless, the reduction in cell specific rates over time suggests a more efficient conversion of available organic carbon into cell biomass, and an increase in substrate quality or nutrient availability for the cells (del Giorgio and Cole 1998). Favourable nutrient and organic C conditions for bacterial growth are known to occur in sea ice as the algal bloom progresses (Becquevort *et al.,* 2009, Kuparinen *et al.,* 2007), which supports our observation of more efficient C conversion at the cell-specific level.

While respiration was relatively constant, BP was highly variable throughout the study and significantly higher in the ice. Sea ice BP values observed in our study covered nearly the entire range reported in the literature (Table 3) and increased by approximately two orders of magnitude over the 3.5-month study, whereas BA only approximately doubled during the same period. This difference between increases in BP and BA suggests that cells were becoming more active over the course of the season, as previously observed by Yager et al. (2001). Indeed, we observed an increase in cell size over time in sea ice combined with increased cell specific BP

rates (Figs 3C and D, respectively). Thus, the concept of larger cells being more active (Gasol *et al.,* 1995) seems to apply to our study.

Table 3 Summary of bacterial variables measured in brine, melted sea ice or crushed ice slurries from published reports and this study. All values are uncorrected for Q10 effect. BP= bacterial production, BA= bacterial abundance, SGR= specific growth rate, TI= thymidine incorporation, LI= leucine incorporation.

Source	Location	Section	Season	BP	Method	BA	Biovolume	Biomass	Gen. time	SGR
				µg C L^{-1} d^{-1}		10^6 cells mL^{-1}	$µm^3$	µg C L^{-1}	d^{-1}	d^{-1}
Bunch and Harland 1990	Frobisher Bay, Canadian Arctic	Bottom 20cm	Winter-Spring	24.96[1]	TI	0.199-1.0	0.65	21.8-43.6	3-294	NM-0.2
Kottmeier et al. 1987a	McMurdo Sound, Antarctic	Bottom 5cm	Summer	0-24.4[2]	TI	0.1-0.5		4.2-96.1	3.5-12	0.06-0.2
Grossmann and Dieckman1994	Weddell sea, Antarctic	Brine	Winter	9.12-10.1	LI	0.02-0.28	0.01-0.57			
Kottmeier et al. 1987b	West of Antarctic Peninsula	Brine (bottom 20cm)	Winter	26.0	TI			17.2-51.3	5.6-8	
Mock et al. 1997	Baltic sea	Brine	Winter-Spring	2.93-5.64	TI	1-2.8	0.2	50-89		
Pusceddu et al. 2008	Gerlache Inlet, Antarctic	Bottom 10cm	Summer	0.087-0.753[2]	LI	0.75-1.29		1-10.2	1.1-4.1	
Kuparinen et al. 2007	Baltic sea (compilation)	Variable	Variable	0.432-25.6	LI			4.6		
Kaartokalio et al. 2007	Baltic sea	Whole core	Spring	0.495[2]	LI	0-1.8	0.078 (0.028-0.138)			1.78
Helmke and Weyland 1995	Weddell Sea, Antarctic	Variable	Winter	0.2-2.7[1]	BA	0.015-2.4	0.065 - >1.33	0.5-157		
This study	Amundsen Gulf, Canadian Arctic	Bottom 5cm	Spring-Summer	0.02-18.3[1]	LI	0.07-0.574	0.08-0.125	1.12-10.4	0.6-53.5	0.019-1.76

[1]Rates measured on melted ice samples

[2]Rates measured on crushed ice slurries

The lack of a relationship between BP and BR suggests a level of uncoupling between both variables, though this result may have been influenced by small sample size or longer incubations for BR estimates. Nonetheless, high variability in BP and more constrained BR resulted in the wide range of potential BGEs, from 0.98 to 74% in sea ice, covering most of the range observed the literature (del Giorgio *et al.,* 1997, del Giorgio and Cole 1998). Not

surprisingly, BP and BGE were strongly related in this study and followed a typical positive hyperbolic function with BP (del Giorgio and Cole 1998, Kritzberg *et al.,* 2005, Roland and Cole 1999). Our extreme BGE values, however, should be interpreted with some caution. The lowest value relates to measures made at the beginning of our sampling time period, when algal biomass was high and the empirically derived proportion of CR attributed to BR was an overestimate resulting in very low BGE estimates. At the other extreme, in the very last sample, when algal biomass was low, the proportion of BR:CR was likely underestimated, resulting in a very high BGE value. Our mean BGE value of 5.8% observed in IWI samples is similar to the reported average of 6.9% in the water column of the Chukchi sea (Kirchman *et al.,* 2009a), but the mean in sea ice was much higher at 24%. The average BGE in sea ice, which suggests a far more efficient conversion of organic carbon into cell biomass seems plausible and is consistent with the more nutrient- and organic-rich environment that sea ice provides, despite methodological limitations. Better quantifying the BR:CR ratio in sea ice will be important in future studies.

At the cell specific level, there was a strong negative correspondence between BGE and BR_{sp} in sea ice, yet if the extreme high and low BGEs are explained by too little and too much respiration attributed to BR, respectively, then not only does this relationship still hold but the slope would be steeper. While no significant linear relationship was observed between BP_{sp} and BGE (Fig. 5A and B), a non-parametric correlation test showed a perfect fit between both variables. One possible explanation is that respiratory needs would decrease in nutrient or DOC rich conditions (Jorgensen *et al.,* 1999), while specific bacterial production would respond nonlinearly, again suggesting differential control of both processes. This negative relationship between BR_{sp} and BGE has been previously reported in lakes, where it was attributed to an increased availability of phosphorus (Smith and Prairie 2004). It is possible that viral lysis may have contributed to phosphorus release during incubations. Indeed, (Middelboe *et al.,* 1996) showed that phosphorus released through viral lysis could significantly increase uptake of DOC in a marine *Vibrio* sp. However, the presence of senescent algae, often observed in sea ice during its the seasonal evolution (Junge *et al.,* 2004, Kottmeier and Sullivan 1988) may also help to explain the observed pattern; i.e. increased labile organic substrates promote BP through increased cell growth and/or a greater proportion of actively growing cells, while BR remains more constrained due to relative constant metabolic respiratory maintenance of dormant cells even when substrates are low.

Given that BP and BA in sea ice were typically higher than in the water column, it is not surprising that BR was also higher, however this increase was proportionally less then that of BP. Furthermore, given the relative difference in the increased rates of BP and BR, higher BGEs were also observed in sea ice. The observation of marked seasonal patterns in sea ice bacterial metabolism supports a strong coupling of bacterial activity with the physical changes during Arctic spring. This coupling could be either direct, by increased rates of metabolism as a function of increases in temperature (Kaartokallio *et al.,* 2007, Mundy *et al.,* 2005), or indirect by increased substrate availability due to enhanced primary production (Becquevort *et al.,* 2009, Bunch and Harland 1990). Both factors may have contributed to the enhanced rates, even though we did not observe a relationship between chlorophyll *a* or nutrients with any of our bacterial variables likely due to small sample size for this study area.

2.4.3 Implications for C cycling and CO_2 fluxes

Sea ice is considerably more permeable to biogenic gases than previously believed (Delille *et al.,* 2007), with biological processing of material, not only changing physical conditions, contributing to the fluxes from sea ice to the atmosphere. Significant atmospheric fluxes of CO_2 from sea ice have been observed in the Amundsen Gulf (Miller *et al.,* 2011), where physical processes alone cannot explain the fluxes measured. Our study shows that community respiration in sea ice may be a significant sink for organic matter and thus, potentially an important source of biogenic CO_2 contributing to these observed atmospheric fluxes.

In order to evaluate net autotrophy (where net PP exceeds respiration) versus net heterotrophy (where respiration exceeds net PP) in sea ice, we compared our measured respiration estimates with previously reported rates of PP from the same region. Using an average rate of sea ice primary production taken for Franklin Bay (Riedel *et al.,* 2008), a productive coastal system in Amundsen Gulf, and comparing it with the average C consumed by CR in our study from beginning of April to mid May, sea ice would be highly net autotrophic with a ratio of CR:PP of 0.11 for the spring bloom period. During this period, polar sea ice may be "biologically" absorbing atmospheric CO_2 (Delille *et al.,* 2007). If we consider other time frames, however, our data indicate net heterotrophy in sea ice: pre-bloom (February to March) estimates of respiration compared to PP rates yielded an average CR:PP ratio of 16 with post-bloom (May to June) ratios being potentially even greater. The latter is based on our high

measured rates of CR as compared to undetectable rates of PP during algal senescence of the post-bloom (Reidel *et al.* 2008). Although sea ice may indeed be net heterotrophic at times, the complexities of carbonate chemistry combined with physical conditions (Fransson *et al.,* 2009, Papakyriakou and Miller 2011, Semiletov *et al.,* 2007) may result in a sea ice system that is ultimately absorbing atmospheric CO_2. The relative importance of biological contributions to the seasonal flux of CO_2 between the atmosphere and the ice is yet to be determined.

2.5 Conclusions

Respiration appears to be an important C sink in Arctic sea ice. Respiration rates were significantly higher in sea ice than in underlying waters and relatively more constrained in sea ice both spatially (across the study region) and temporally (from March to July). BP showed considerably higher temporal variability, which drove the wide range of observed BGE. At the cell-specific level, there was a negative relationship between BGE and BR_{sp} in sea ice, which may be a function of decreased respiratory demands under conditions of increased nutrient availability. Microbial metabolic processes in polar waters may be particularly vulnerable to environmental changes and warming temperatures (Kirchman *et al.,* 2009b) but changes in sea ice dynamics will also impact bacterial metabolism. Furthermore, studies at lower latitudes have experimentally shown a differential response between BR and BP to temperature increases, where BR increases at a much faster rate then BP, particularly when initial temperatures are low (Apple *et al.,* 2006, Hoppe *et al.,* 2008) Furthermore, community respiration may respond more strongly than PP to increasing temperatures in the Arctic (Vaquer-Sunyer *et al.,* 2010), potentially enhancing the trend towards net heterotrophy. Thus, although many have emphasized how Arctic warming and reduced ice cover will favour higher PP (Arrigo *et al.,* 2008, Pabi *et al.,* 2008, Wassmann *et al.,* 2008), our study of respiration and carbon cycling adds to the evidence that an increasing proportion of PP will be lost via respiration (Vaquer-Sunyer *et al.,* 2010). The relative role of sea ice respiration in C cycling and the biological contribution to atmospheric gas exchange in a warming Arctic Ocean remain open questions.

2.6 Acknowledgements

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Chapitre 3 : Respiration et dynamique bactérienne du carbone dans le Golfe d'Amundsen, Arctique Canadien de l'Ouest

Photo : Traversée de crêpes de glace au large de l'île de Banks, Golfe d'Amundsen, Mars 2008

Respiration and bacterial carbon dynamics in the Amundsen Gulf, western Canadian Arctic

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Abstract

Respiration rates are fundamental to understanding ecosystem C flux, however respiration remains poorly characterized in polar oceans. The Circumpolar Flaw Lead (CFL) study provided a unique opportunity to sample the Amundsen Gulf, from November 2007 to July 2008 and follow microbial C dynamics. This study shows that bacterial production (BP) was highly variable, ranging from 0.01 to 2.14 μg C L^{-1} d^{-1} (CV = 192%), whereas the range in community respiration (CR) was more conservative from 3.8 to 44.2 μg C L^{-1} d^{-1} (CV = 55%), with measurable rates throughout the year. The spring-summer peak in BP preceded the peak in CR suggesting differential predominant control. From May until July, BP was more related to chlorophyll *a* concentration (r=0.68) whereas CR was not. Given the observed high variability, BP was the main driver of bacterial growth efficiency (BGE) (r^2 = 0.86). The overall average BGE was low at 4.6%, ranging from 0.20 in winter to a peak of 18.6% during the spring bloom. This study confirms that respiration is an important fate for C in the Amundsen Gulf and our rate-based estimates of ecosystem scale CR suggests that considerably more C is respired than could be accounted for by gross primary production (GPP). One of the most plausible explanations for this observed discrepancy is that regenerated primary production is currently underestimated.

3.1 Introduction

One of the dominant fates of organic carbon (OC) and a fundamental process in aquatic ecosystem metabolism is the transformation of OC to inorganic C (CO_2) via the respiration of organisms. From a trophodynamic perspective, respiration is considered a C sink as it represents an important loss of OC from the ecosystem (del Giorgio and Duarte 2002, Jahnke and Craven 1995). In most aquatic systems, microbes, primarily heterotrophic bacteria, are responsible for the majority of respiratory losses: 45%, on average, for the global ocean (Robinson 2008) with a range of 25 to 90% of community respiration (CR) being attributed to bacterial respiration (BR) alone in polar and sub-polar regions (Kirchman *et al.,* 2009a, Rivkin and Legendre 2001, Robinson *et al.,* 1999, Sherr and Sherr 1996). Despite this important functional role, there are considerably fewer estimates of respiration by comparison to rates of primary production (PP) in the literature (Williams and del Giorgio 2005), particularly in polar oceans, representing an important knowledge gap in the biogeochemical cycling of C.

Another important functional role of bacteria is bacterial production (BP), the conversion of dissolved organic C (DOC) into biomass for subsequent transfer to the food web (Azam *et al.,* 1983, Pomeroy 1974). The relative amount of C used for biomass production versus the total amount consumed for metabolism (both production and respiration) is referred to as bacterial growth efficiency (BGE, where BGE=BP/(BP+BR) (del Giorgio and Cole 1998). BGE is highly variable ranging from less than 1% to 65% across marine ecosystems, with an average C conversion efficiency of approximately 15% for open oceans (del Giorgio and Cole 2000). As resource limitation often results in decreased BP while having only negligible effects on BR, BGE typically increases with higher productivity and inorganic nutrient availability (Robinson 2008). BP has also been more commonly measured in the global oceans and the total amount of C estimated to flow through bacteria is typically converted using either fixed estimates of BGE or BR estimates empirically derived from measured BP (del Giorgio and Cole 1998, 2000, Rivkin and Legendre 2001).

One region where there is very little information available on the amount of C lost through microbial respiration or the characterization of BGE is the Arctic Ocean, where only a handful of respiration measurements from different regions exist (Cottrell *et al.,* 2006, Kirchman *et al.,* 2009a, Kritzberg *et al.,* 2010, Sherr and Sherr 2003, Vaquer-Sunyer *et al.,* submitted).

Working in the Arctic poses a great logistical challenge not only in terms of access to sites on a regular basis but also of the technical challenge of making accurate microbial rate measurements. Conditions also vary widely in polar seas on both a temporal and spatial basis: from complete darkness to full sunlight, from little autochthonous production to very high rates of primary production (PP) resulting in large pulses of labile C, a wide range of surface temperatures, changing sea ice conditions and areas with and without significant riverine OC inputs. Thus it is difficult to elucidate how microbial respiration, and the relative fate of OC, will change over space and time.

The Arctic Ocean is one of the most rapidly changing ecosystems on earth (Wassmann 2011). Increased temperatures as a function of climate change have resulted in a dramatic loss of permanent sea-ice cover and the thinning of sea ice (Comiso *et al.,* 2008, Maslanik *et al.,* 2007). This reduction in sea ice coverage has increased the relative amount of open water and has extended the phytoplankton growing season, resulting potentially in a greater amount of C inputs via primary production (Arrigo and van Dijken 2011, Wang *et al.,* 2005). Riverine input of terrestrial OC is expected to increase due to the melting of the permafrost (Peterson *et al.,* 2006) potentially increasing the terrestrial OC load. Sea surface temperatures during the summer are also rising (Comiso 2003). Increased autochthonous and allochthonous C inputs and rising water temperatures should also impact the rates of microbial respiration, and the relative conversion efficiency of bacteria (BGE; del Giorgio and Cole, 1998). In contrast to PP (e.g. Arrigo and van Dijken, 2011), there is no comparable baseline for respiration and bacterial C conversion efficiency in Arctic waters. C cycling throughout the Arctic in general will be greatly altered with climate change (Kirchman *et al.,* 2009b, McGuire *et al.,* 2009).

The Circumpolar Flaw Lead (CFL) system study provided a unique opportunity to characterize the seasonal patterns of bacterial production, respiration and BGE over a nine-month period in the Amundsen Gulf in the southeastern Beaufort Sea (Barber *et al.,* 2010, Deming and Fortier 2011). This study enabled us report the seasonal patterns of measured rates of BP and CR and BGE, and elucidate some of the main controlling factors. Furthermore it allowed us to estimate how much C was fluxing through microbial communities, thus providing novel information on the seasonal respiratory demands and microbial C cycling dynamics of this particular region of the Arctic Ocean.

3.2 Material and methods

3.2.1 Study site

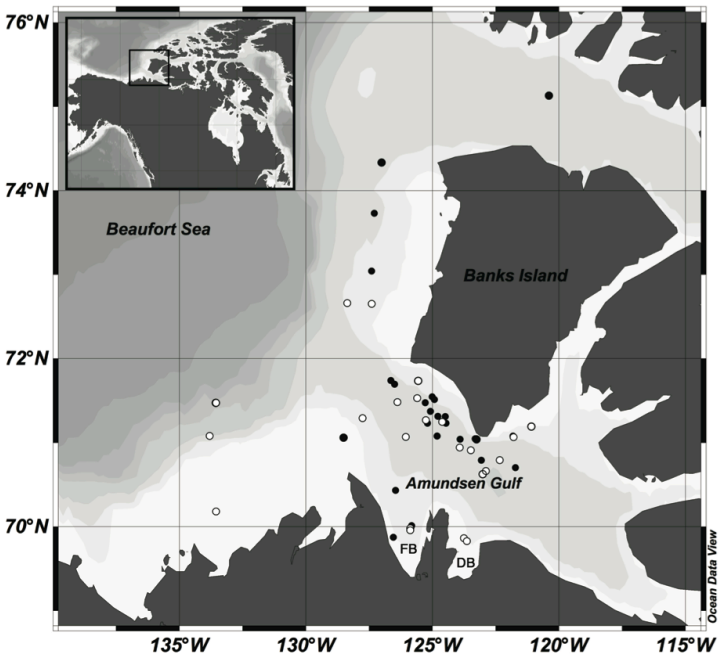

Figure 1. Map of the area sampled in the Amundsen Gulf, eastern Beaufort Sea and M'Clure Strait. Filled circles denote stations sampled for bacterial production only and open circles, stations sampled for both bacterial production and respiration. FB and DB denote Franklin and Darnley Bay, respectively. Inset shows map of Canada for spatial reference.

From November 2007 to July 2008, sampling was carried out onboard the *CCGS Amundsen* in the Amundsen Gulf of the south eastern Beaufort Sea in the western Arctic Ocean (Figure 1), as part of the International Polar Year–Circumpolar Flaw Lead system study (IPY–CFL). A total of 45 stations were sampled (Figure 1), for a total of 50 discrete measurements of respiration and 270 of BP. This study includes different periods of the year: (1) the fall sea ice freeze-up, (2) the winter maximum ice cover, (3) the vernal sea ice break-up and (4) the summer open water season. For a comprehensive review of the basis of the CFL project and general physical conditions during the study, see Barber *et al.* (2010).

3.2.2 Sample collection and processing

Every 7 to 14 days, water samples were collected at four to six depths using a Carousel Rosette equipped with twenty-four 12 L Niskin-type bottles. Depths of interest were the surface (<12 m), nitracline (15-90 m), chlorophyll maximum when present (10-71 m), O_2 minimum (120-253 m) and occasionally at bottom depths (125-967 m). BP was measured at all 6 depths per cast, and 2 to 3 were selected for respiration measurements, with 1 depth being the surface and the

other most often at the nitracline and chlorophyll max, however rarely at depths below 100m. The choice of sampling depths was based on the potential presence of interesting biological gradients at any given depth. The criteria for selection varied of the course of the year, following changes in irradiance and primary productivity in spring and summer. Water was gravity-filtered directly from the Niskin-type bottles using 53 µm Nitex mesh to remove large zooplankton. Water was kept in the dark in isothermal containers until processing onboard.

3.2.3 Physical and chemical variables

Profiles of temperature and salinity were obtained using a SeaBird 911+ CTD mounted on the rosette, with additional probes for oxygen and chlorophyll fluorescence. Concentrations of phosphate (PO_4), silicic acid ($Si(OH)_4$) and nitrate+nitrite ($NO_3^-+NO_2^-$) and nitrite (NO_2^-) were determined using an Bran and Luebbe Autoanalyzer 3 with routine colorimetric methods [*Grasshoff et al.*, 1999]. $NO_3^-+NO_2^-$ is hereafter referred to as NO_3^-, as NO_2 concentrations were minimal throughout the entire period. Detailed analytical methods for nutrient analyses are reported elsewhere by (Martin *et al.,* 2010, Simpson *et al.,* 2008).

3.2.4 Bacterial abundance, cell biovolume and bacterial biomass

We use the term "bacteria" to refer to both archaea and bacteria, as the latter is usually the dominant prokaryotic group in Arctic waters (Garneau *et al.,* 2008, Kirchman *et al.,* 2007) and in order to lighten the text. Bacterial abundance (BA, cell mL^{-1}) was determined using epifluorescence microscopy of DAPI (4'6'-Diamidino-2-phenylindole dihydrochloride) stained cells (Porter and Feig 1980). Briefly, 20 mL water samples were fixed with 1 mL formaldehyde (37%) and stored in the dark at 2-4 °C until processing. Samples (10-20 mL) were filtered on 0.2 µm polycarbonate black filters (25 mm, Millipore) to which 50 µL of DAPI (0.5 mg mL^{-1}) stain was added. The stain was left on the filter for 5 minutes before completing the filtration. Towers were rinsed with 5 mL of ultrapure water to minimize bacterial adhesion to tower walls and distribute cells evenly on the filter. Filters were mounted on slides and stored at -20°C until photographed using a digital camera (Canon Canada) mounted on a Leica epifluorescence microscope. Counts were done by image analysis using the ImageJ software (Abramoff *et al.,* 2004). For each slide, 7 fields or more were photographed and analyzed, and a minimum of 200 cells was counted for each slide. From these images, cell biovolume (V, μm^3) was calculated using the equation $V = 4\pi r^3/3 + (l - 2r)\pi r^2$, assuming a cylinder shaped cell with two

hemispherical caps (Smith and Prairie 2004). Spherical cells occur when $l = r$. Presented values are the mean cell volume in a given sample. All size measurements were calibrated against fluorescent microspheres (ThermoFisher Scientific) to account for halo effect. Cells with a volume lower than 0.00418 μm^3 were excluded (corresponding to a pore size of 0.2 µm) and large cells with a volume greater than 0.344 μm^3 were not considered bacterial (Gasol *et al.,* 1995, Straza *et al.,* 2009) The mean C content per cell (CC, fg $cell^{-1}$) was calculated using a conversion factor of 148 fg C μm^{-3} (Kirchman *et al.,* 2009a).

3.2.5 Bacterial production

Bacterial production was measured using the ^{3}H-leucine incorporation method (Smith and Azam 1992). Water samples (1.2 mL) were dispensed, in triplicate, into clean 2 mL microcentrifuge tubes pre-loaded with 50 µL ^{3}H-leucine (115.4 Ci $mmol^{-1}$, Amersham) to produce a final leucine concentration of 10 nM (Garneau *et al.,* 2008). Samples were incubated in the dark for approximately 4 h at in-situ temperature. Leucine incorporated into cell protein was collected after precipitation with 250 µL 50% trichloroacetic acid (TCA) and centrifugation at 14000 RPM where the protein adhered to tube walls. The liquid was aspirated and samples were rinsed with 5% TCA with a second centrifugation and aspiration. Tubes were filled with 1.25 mL liquid scintillation cocktail (ScintiVerse, Fisher Scientific) and radioactivity was measured using a Tri-Carb 2900 TR Packard Liquid Scintillation Analyzer. Rates of leucine incorporation were corrected for radioactivity adsorption using TCA killed controls and converted to bacterial C production (BP) using a conversion factor of 1.5 kg C per mol ^{3}H-leucine (Garneau *et al.,* 2008). Cell specific BP (BP_{sp}) was calculated as the ratio of BP to BA.

3.2.6 Respiration rate and potential bacterial growth efficiency

Samples for community respiration (CR) measurements were allowed to equalize with incubation temperature in the ship's cold room prior to transfer in 500 mL gas-tight glass Erlenmeyers. CR was calculated by measuring the change in dissolved O_2 consumption with optical fiber optodes (Fibox, PreSens, Germany) adapted from methods described by (Kragh *et al.,* 2008, Marchand *et al.,* 2009). Briefly, an O_2 sensitive sensor spot (pst3, 5 mm) is fixed to the internal wall of a 500 mL Erlenmeyer flask in which the samples are incubated. O_2 concentrations are measured by linking a light emitting (600-660 nm) optical fiber to the sensor spot (from the outside of the bottle), and the sensor emits more or less luminescence depending on O_2

concentrations in the sample. Measurements account for salinity and O_2 concentrations are corrected for sample temperature. Unlike other methods, the use of optodes has the advantage of leaving the incubation unperturbed with almost real time O_2 measurements, while being relatively space efficient and not producing toxic waste. The limit of detection of the method is 0.03% $O_{2\ sat}$ with a relative accuracy of +/- 0.4% at 20.9%$O_{2\ sat}$.

Incubations for CR were carried out in duplicate for 5 to 10 days in a temperature controlled cold room and Erlenmeyers were kept in the dark submerged under water in isothermal containers to minimize temperature variations and prevent gas exchange. O_2 concentrations were measured at 24 to 48 h intervals. For each station, a 500 mL Erlenmeyer flask containing only ultrapure water was used as control. In all incubations, O_2 concentrations in the control were either stable or increased slightly, suggesting possible underestimation of CR rates.

Temperature of the chamber was kept at 2-4°C during all incubations and remained stable over the course of the incubation period. The difference between incubation and *in situ* temperature was +4.3°C on average, with a range of ± 0-6°C with 70% of the samples incubated within a narrow range of 4-5.4°C warmer than *in situ* (see Table 1 SI and Figure 1 SI). Only 4 samples were incubated at temperatures lower than *in situ*. Although it is preferable to keep incubations as close as possible to *in situ* temperature, logistical and material constraints did not permit this.

To correct for temperature deviations from *in situ*, we applied a metabolic conversion factor commonly referred to as Q_{10} to derive more plausible *in situ* CR rates. We used a Q_{10} value of 4 based on average Q_{10} measurements made specifically in the polar environments and under cold-water conditions for CR (Apple *et al.,* 2006, Kritzberg *et al.,* 2010, Martinez 1996, Vaquer-Sunyer *et al.,* 2010, Yager and Deming 1999). Although higher than the canonical Q_{10} conversion factor of 2, given that our incubation temperatures were higher than *in situ* over 90% of cases, the use of a Q_{10} of 4 provided a more conservative estimation of CR. Values were converted to C respired ($\mu g\ C\ L^{-1}\ d^{-1}$) assuming a respiratory quotient of 0.8 (Robinson *et al.,* 1999, Williams and del Giorgio 2005). Linear rates were observed throughout the incubation period and support a limited bottle effect, as suggested in a recent review (Robinson 2008). For quality control, any incubation with an r^2 inferior to 0.60 was rejected and the mean r^2 during incubation periods was 0.90.

Other than a 53 µm Nitex mesh gravity screening at the rosette, no additional filtration was performed therefore whole community respiration (CR) rates were determined. Previous studies have shown that polar bacterial communities can be strongly associated to particles (Garneau *et al.,* 2009, Kellogg *et al.,* 2011). Given the anticipated low respiration rates in Arctic waters and the potential importance of particle-associated bacteria, we omitted a pre-filtration step as that may have compromised our ability to measure any rate of respiration.

Bacterial respiration (BR) was therefore estimated using an empirically derived equation reported by Robinson (2008), where $BR=0.45CR^{0.93}$. This relationship is based on successful fractionation experiments carried out in various locations in the global ocean. This equation allows for variations in the CR:BR ratio, and results in a lower relative proportion of BR at high CR rates. Using this equation, the mean BR:CR ratio during this study was 44% and ranged from 41 to 49%. Kirchman *et al.,* (2009a) observed lower proportions in the Chukchi Sea with an average of BR:CR of 25% when he excluded high values where BR > CR. However, when we include these high estimates, assuming a 100% value, the average BR measured by Kirchman *et al.* (2009a) increases to 44% of CR. While we acknowledge that in some cases our BR may be overestimated (or underestimated), the empirical conversion model of Robinson (2008) is currently the most robust option. BGE estimates were calculated as the ratio of BP to (BP+BR). Several conversion factors were used to estimate CR, BR and therefore BGE. For this reason we consider these all to be potential estimates, but we will refer to them simply as CR, BR and BGE for the remainder of the text.

3.2.7 Phytoplankton biomass

Samples for phytoplankton chlorophyll *a* (Chl *a*) biomass and pheopigment concentration were filtered on 25 mm diameter Whatman GF/F filters (nominal pore size 0.7 µm). Filters were then placed in 90% acetone over 24 h at 5°C in the dark for pigment extraction. Fluorescence of the extracted pigments was measured using a Turner Designs fluorometer model 10-AU [acidification method: Parsons *et al.*, 1984). Chl *a* and pheopigment concentrations were then calculated using equations of Holm-Hensen *et al.* (1965).

3.2.8 Statistical analysis

Type 1 linear regressions and non-linear relationships were calculated using SigmaPlot 10 (Systat Software Inc) for Windows. All linear regressions excluded samples collected at coastal

sites during the spring, namely Franklin and Darnley bays, since our main objective was to follow microbial dynamics associated with flaw leads, occurring away from the coasts. Coastal sites were sampled only in May and June, and were characterized by significant allochthonous spring inputs from rivers and coastal runoff and lower water depths (<100 m), and were typically outliers in our general trends. Student's t-test for difference of means and descriptive statistics were computed with PASW 18.0 for MacOSX (SPSS Inc). Volumetric data were converted to areal data using standard trapezoidal integration over the top 80 m of the water column. This depth interval was chosen based on mean euphotic zone depth and covered most of the phytoplankton production realized in the water column (Forest *et al.,* 2011). When necessary, data were log-transformed to meet normality and homoscedasticity assumptions of parametric tests.

3.3 Results

3.3.1 Seasonal patterns in bacterial dynamics

Table 1. Seasonal means and ranges of variables measured in Amundsen Gulf for all samples collected at variable depths in the water column[a].

Variable	November to April				May to July				Overall	
	Mean	Range	SD	*n*	**Mean**	Range	SD	*n*	**Mean**	SD
BP (μg C L^{-1} d^{-1})	0.053*	0-0.242	0.05	182	0.358*	0.004-2.68	0.44	87	0.146	0.29
CR (μg C L^{-1} d^{-1})[b]	11.1*	3.8-22.5	5.0	21	18.7*	6.7-44.2	9.0	29	15.5	8.43
BR (μg C L^{-1} d^{-1})[bc]	4.94	1.84-9.64	2.1	21	8.05	3.14-18.1	3.6	29	6.74	3.41
BGE (%)[bc]	1.82*	0.20-5.63	1.6	21	6.60*	0.44-18.6	5.3	29	4.59	4.82
BA (x10^5 cells mL^{-1})	1.63*	0.77-2.48	0.40	39	3.9*	0.72-12.3	2.7	37	2.73	2.20
Biovolume (μm^{-3} $cell^{-1}$)	0.107	0.055-0.156	0.020	39	0.103	0.064-0.150	0.021	37	0.105	0.020
BB (μg C L^{-1})	2.6*	0.85-4.9	0.87	39	5.5*	1.1-16.9	3.4	37	4.02	2.85
BP_{sp} (fg C $cell^{-1}$ d^{-1})	0.39*	0.02-1.1	0.33	33	1.59*	0.186-5.42	1.20	29	1.02	1.08
BR_{sp} (fg C $cell^{-1}$ d^{-1})[bc]	32.8	9.6-60.0	22.0	21	31.2	6.81-95.0	31.2	29	31.8	22.0
Chl *a* (μg L^{-1})	0.09*	0-4.4	0.41	116	1.46*	0.001-10.6	2.31	59	0.54	1.50
Pheopigments (μg L^{-1})	0.06*	0.01-0.27	0.04	116	0.33*	0-2.2	0.41	54	0.15	0.26
NO_3^- (μmol L^{-1})	10.3	0-20.1	6.52	153	8.88	0-22.6	6.42	81	9.82	6.51
PO_4^{3-} (μmol L^{-1})	1.15	0.44-1.9	0.39	52	1.03	0-1.69	0.44	58	1.09	0.42

[a]Values from November to April, May to July and the overall study period are presented. Depth range was concentrated from 0-100m. The asterisk denotes significant differences between periods ($p \leq 0.05$) according to Student's t-test for differences of means.

[b]Q_{10} corrected values

[c]Based on the equation $BR=0.45CR^{0.93}$ [*Robinson*, 2008]

Seasonal patterns for BP, CR, BA, and BGE are presented in Figures 2A-D. BP rates were very low and stable from November until mid-April (Figure 2A). Rates of BP became much higher and slightly more variable from mid-May to July (Figure 2A), with a coefficient of variation (CV) from the late spring-summer of 123% as compared to 99% from winter-early spring (Table 1). Rates of CR were measured throughout the entire sampling period (Figure 2B), and were on average 210 times higher than BP from the winter-early spring period and 52 times higher in the late spring-summer. Compared to winter-early spring, average CR rates increased in late spring-summer by a factor of 1.7 but this was markedly less than BP that increased by a

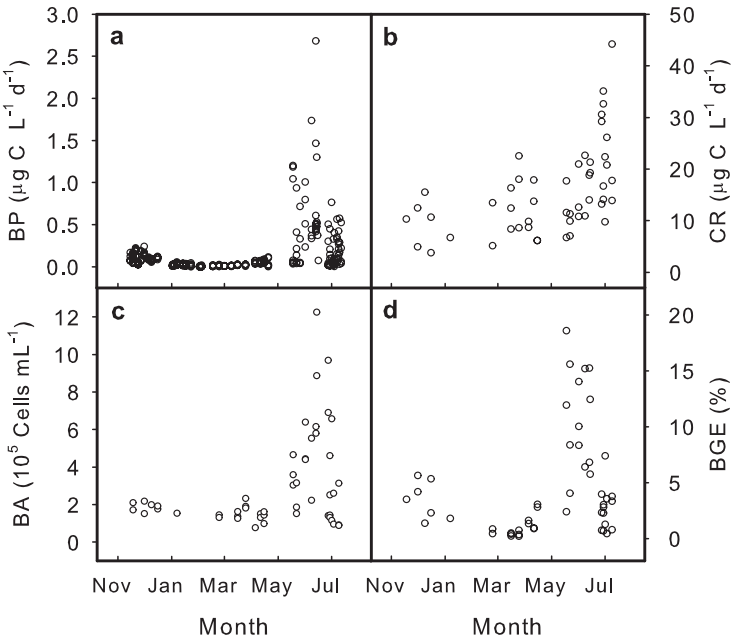

Figure 2. Temporal pattern of measured rates of A) bacterial production (BP), B) community respiration (CR), C) bacterial abundance (BA) and D) bacterial growth efficiency (BGE) sampled from November 2007 until July 2008.

factor of 6.8 on average. BA also followed a similar pattern to both BP and CR with low constant abundance throughout the winter and higher more variable numbers in the spring-summer (Figure 2C). Overall biomass increased as well, but this was solely as a function of an increase in abundance, as we observed no measurable difference in cell biovolume between cells sampled in winter and in summer (Table 1).

Converting CR into BR using the equation of Robinson (2008) enabled us to look at cell specific respiration rates and allowed us to compare how this varied over the sampling period. The relative increase in abundance of 2.4 was higher than that of BR, making for an interesting

contrast in seasonal cell specific rates. Cell specific rates of bacterial respiration (BR_{sp}) were not significantly different between the sampling periods at around 30 fg C $cell^{-1}$ d^{-1}, whereas cell-specific rates of BP (BP_{sp}) were significantly higher in late spring-summer (Table 1). This stability in average respiration per cell between the seasons was a surprise, however the

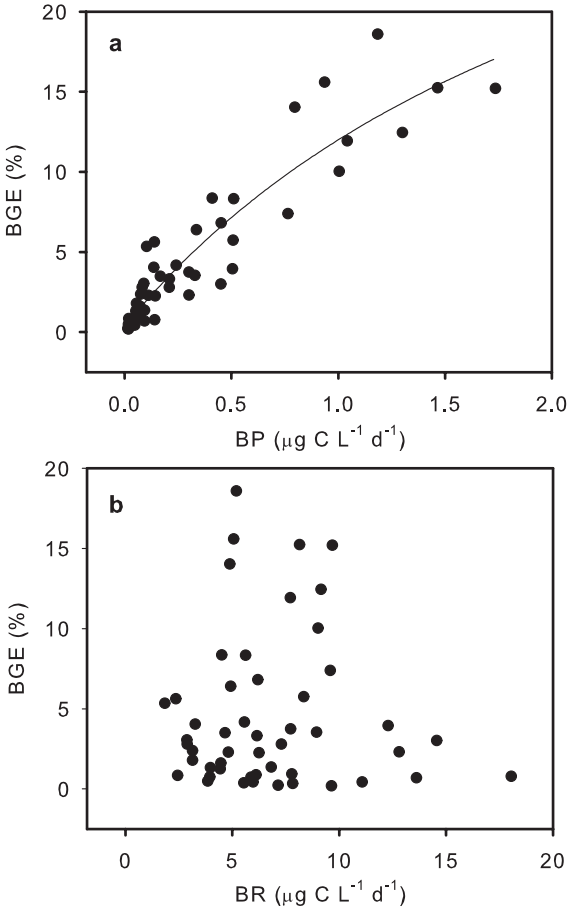

Figure 3. Overall relationship of BGE modelled A) as a hyperbolic function of bacterial production (BP) ($r^2 = 0.86$); modelled equation reported in Table 2 and B) as a function of bacterial respiration (BR) (non-significant).

variability in the BR_{sp} in late spring-summer was much greater with values increasing toward the end of our sampling period.

The BR estimates allowed for the calculation of BGE, which was lower and more stable in winter-early spring as compared to the late spring-summer (Fig. 2D). Given that BP was more variable over the whole sampling period, with a CV of 192% as compared to a more constrained annual variability in BR (CV=55%), changes in BGE were driven by changes in BP. Indeed the overall relationship between BP and BGE, best described using a positive hyperbolic function, was very strong (Table 2, Figure 3A, $r^2 = 0.86$). No significant relationship was observed between BGE and BR (Figure 3B).

3.3.2 Factors controlling BP and CR

Despite an apparent similar seasonal trend, no significant relationship was observed between BP and CR estimates, suggesting that these rates were controlled, at least primarily, by different factors. Volumetric rates of BP could be predicted from Chl *a* concentrations throughout the entire sampling period by a moderately strong positive relationship explaining 43% of the variation in BP (Table 2, Figure 4A). No significant relationship was observed between CR and Chl *a* (Table 2, Figure 4B).

In the overall relationships, CR was significantly and linearly related to water temperature and to NO_3^-, the latter as a log-log function, explaining 57% and 26% of the variance, respectively (Table 2). BP, however, was not significantly related to temperature but was weakly related to NO_3^- ($r = -0.38$), also in a log-log relationship. When the sampling period was restricted to late spring-summer only, relationships with CR were slightly stronger. The relationship with temperature explained 61% of the variability in CR (Table 2) but the latter should be interpreted with caution given the Q_{10} conversion factors used to derive estimates. In the case of NO_3^-, relationships with BP and CR were stronger during the summer explaining 33% and 47% of the variation respectively (Figure 5A and B, Table 2). The summer relationship between BP and Chl *a* remained similar to the overall data ($r = 0.68$) and even when restricted to this period, there was still no observed significant relationship with CR.

The uncoupling of BP and CR could also be inferred by a time lag as observed in our time-series and in integrated measured rate values, averaged by seasons. Rates of CR were on average 1.8 times higher in late June-July as compared with late March to early June, whereas for BP, rates were 1.4 times lower. BP responded earlier and more strongly to the accumulation of algal biomass and the concomitant labile C pulse. Increase in CR however occurred later in the slightly warmer waters in late June and July, when BA was highest. This was observed after the peak in production and when NO_3^- was depleted in the post-bloom period.

Table 2. Parameter estimates and statistics of regression models for community respiration (CR), bacterial production (BP) and BGE modeled with other variables[a].

Variable		Regression Model function	*n*	*p*	*r*
Overall					
CR (µg C L^{-1} d^{-1})					
	BA (cells mL^{-1})	CR=2.5E-05BA+9.51	41	0.0053	0.51
	Chl *a* (µg L^{-1})	NS	33		
	Temperature (°C)	CR= 2.9 T+16.7	42	0.0000	0.77
	Nitrate (µmol L^{-1})	LOG(CR)=-0.20LOG(NO_3)+1.18	34	0.0015	-0.53
BP (µg C L^{-1} d^{-1})					
	BA (cells mL^{-1})	LOG(BP)=1.21LOG(BA) -7.49	58	0.0002	0.47
	Chl *a* (µg L^{-1})	LOG(BP)=0.44LOG(Chl *a*)-0.65	159	0.0001	0.66
	Temperature (°C)	NS	233		
	Nitrate (µmol L^{-1})	LOG(BP)=-0.35LOG(NO_3)-2.59	190	0.0001	-0.38
BGE (%)					
	BP (µg C $L^{-1}d^{-1}$)	BGE=0.36+(40.9BP/(2.52+BP))	51	0.0000	0.93
	Chl*a* (µg L^{-1})	BGE=2.52Chl *a*+5.5	33	0.0033	0.50
	Temperature (°C)	NS	42		
	Nitrate (µmol L^{-1})	NS	40		
May to July					
CR (µg C $L^{-1}d^{-1}$)					
	BA (cells mL^{-1})	NS	20		
	Chl *a* (µg L^{-1})	NS	18		
	Temperature (°C)	CR= 2.7T+ 18.0	20	0.0000	0.79
	Nitrate (µmol L^{-1})	LOG(CR)= -0.22LOG(NO_3)+1.27	16	0.0029	-0.71
BP (µg C $L^{-1}d^{-1}$)					
	BA (cells mL^{-1})	LOG(BP)=0.72LOG(BA)-4.5	23	0.017	0.49
	Chl *a* (µg L^{-1})	LOG(BP)=0.47LOG(Chl *a*)-0.61	49	0.0001	0.68
	Temperature (°C)	NS	64		
	Nitrate (µmol L^{-1})	LOG(BP)= -0.56LOG(NO_3)-0.51	51	0.0001	-0.59
BGE (%)					
	BP (µg C $L^{-1}d^{-1}$)	BGE =-0.32+(39.8BP/(2.2+BP))	29	0.0000	0.92
	Chl *a* (µg L^{-1})	NS	18		
	Temperature (°C)	NS	20		
	Nitrate (µmol L^{-1})	NS	20		

[a]Relationships for the overall study period and late spring-summer (May-July) are shown. Bacterial respiration, BR; bacterial production, BP; bacterial abundance, BA; bacterial growth efficiency, BGE; chlorophyll *a,* Chl *a*; temperature, T; nitrate, NO_3; number of samples, *n*; *p* values, *p*; Pearson correlation coefficient, *r*; NS: not statistically significant.

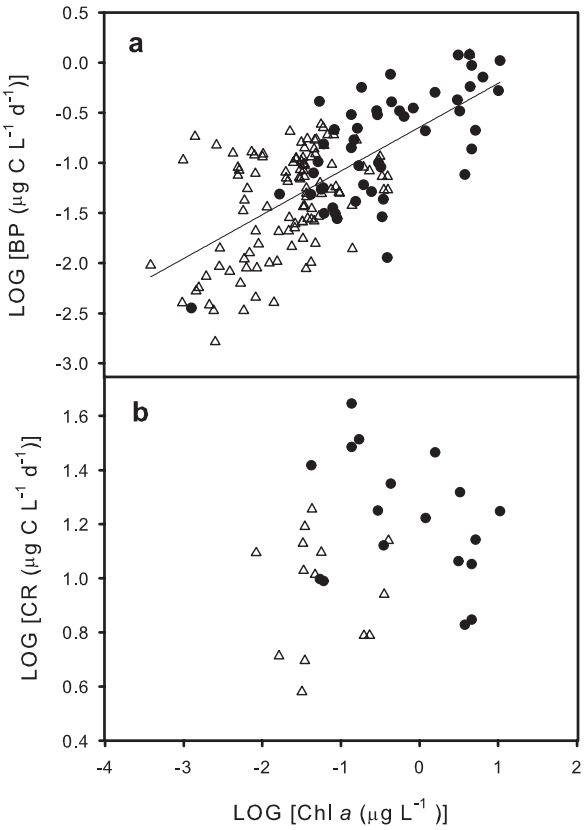

Figure 4. Overall log-linear relationships between chlorophyll *a* (Chl *a*) concentration and A) bacterial production (BP) (r = 0.66; equation presented in Table 2) and B) community respiration (CR) (non-significant). The November-April period is represented by open triangles and the May-July period by full circles.

3.4 Discussion

3.4.1 Seasonal patterns and controls of CR, BP, and BGE

The seasonal estimates of CR, BP, and BGE in this study represent a unique contribution to our understanding of microbial C metabolism for the Amundsen Gulf specifically and the Arctic Ocean in general. Few studies have attempted to measure respiration in the Arctic and generally considered short seasonal observation windows and only a subset of relevant bacterial activity parameters (Table 3). In this study, the concomitant estimates of CR, BP, and BGE obtained over a nine month consecutive time period thus represent a unique contributionto our understanding of microbial C metabolism for the Amundsen Gulf specifically and the Arctic Ocean in general.

Over the course of this study, BP was the most variable rate ranging over 2 orders of magnitude. Our average values are similar to those reported in other Arctic studies but our

extremes covered a slightly wider range (Table 3). The seasonal pattern we observed was similar to the one observed in Franklin Bay (Garneau *et al.,* 2008), adjacent to the Amundsen Gulf during another overwintering program in 2004, where low levels of BP were sustained in the winter and rates increased with the spring bloom. We also observed a strong relationship with Chl *a*, as reported by others for several regions of the Arctic (Garneau *et al.*, 2008; Kirchman *et al.*, 2009a). We saw no statistically significant effect of water temperature on BP, possibly as a result of the limited range of temperatures observed during this study. This may also suggest a co-limitation with organic matter availability (Kirchman *et al.,* 2009b, Pomeroy and Wiebe 2001). This may be a function of the earlier ice breakout that occurred during the CFL study (Barber *et al.*, 2010) that resulted in a slightly earlier peak in algal production (Forest *et al.*, 2011). Algal blooms do not necessarily coincide with warmer surface temperatures in the Arctic (Tremblay *et al.,* 2006b), although in some years they might. Alternatively, the response of BP to additional substrates may have been so strong that any temperature effects were masked. Regardless, it is clear that the growth of Arctic bacterial communities responds almost unilaterally to substrate additions (Cuevas *et al.,* 2011, Meon and Amon 2004, Yager and Deming 1999), while the effect of temperature in stimulating

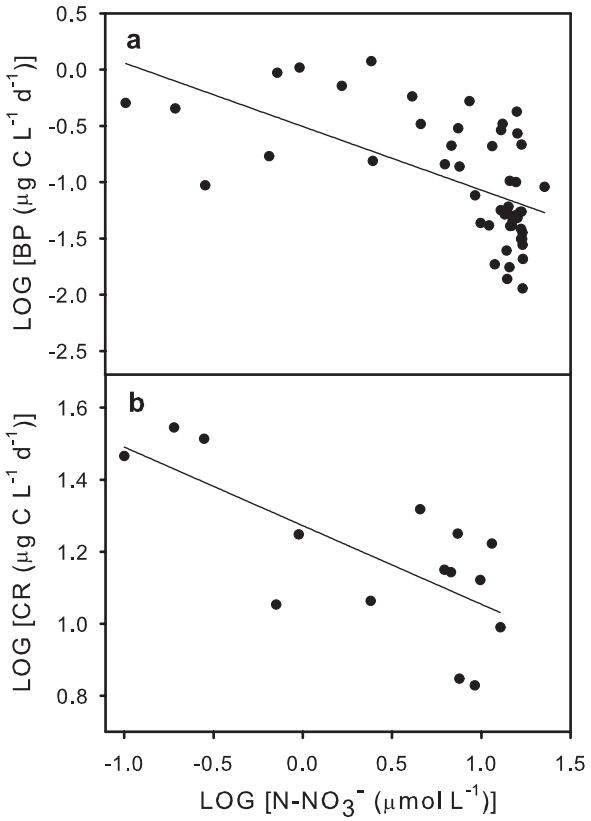

Figure 5. Negative log-linear relationship between A) bacterial production (BP) and B) community respiration (CR) and nitrate ($N\text{-}NO_3^-$) concentration ($\mu mol\ L^{-1}$) for the May-July period. Details are presented in Table 2.

bacterial growth without the addition of substrates in the Arctic remains unclear (Kirchman *et al.,* 2005, 2009b).

CR followed a similar pattern to BP: there were low but measurable levels in the winter, increasing in the spring and summer. However the following important differences were observed. First, measured CR rates and estimated BR were considerably higher than BP and a very high proportion of the total C demand. This is also evidenced by the low BGEs in the winter, within the lower range of published values, but similar to other winter values reported from the Arctic. This suggests that a far greater amount of OC is respired in the Amundsen Gulf than previously thought (Garneau *et al.*, 2008). The high CR rate estimates in our study are well within the range of published respiration reports for other Arctic regions (Table 3), further substantiating our values and supporting the notion that respiration is an important C sink in the polar regions.

In addition, the peak in respiration followed the peak in BP. This temporal uncoupling resulted in a lack of a relationship of CR with BP and Chl *a*. This uncoupling between BP and CR is not surprising and has often been observed between BR and BP (del Giorgio *et al.,* 1997, del Giorgio and Cole 1998, Maranger *et al.,* 2005), while the temporal shifting of peaks in time-series is not commonly reported. We did however see a correspondence of CR with temperature in summer, however our relationship must be interpreted with caution, because of the conversion factors used to derive CR estimates. Nevertheless a strong effect of temperature on microbial respiration has certainly been observed in the Arctic and elsewhere (Apple *et al.,* 2006, Hoppe *et al.,* 2008, Kritzberg *et al.,* 2010, Rivkin and Legendre 2001, Vaquer-Sunyer *et al.,* 2010, Vosjan and Olanczukneyman 1991, Yager and Deming 1999). Moreover, although some studies have observed an increase in both BP and CR with temperature, typically respiration responds more strongly (Kritzberg *et al.,* 2010, Rivkin and Legendre 2001, Vaquer-Sunyer *et al.,* 2010) which will have serious implications for the metabolic balance of the system in a warming climate (Kirchman *et al.,* 2009b, Vaquer-Sunyer *et al.,* submitted, Wohlers *et al.,* 2009). This remains an important line of future research.

CR rates were by comparison less variable than rates of BP. The relatively more conservative range in CR as compared to BP could suggest a more limited effect of change in substrate availability on CR (Lopez-Urrutia and Moran 2007, Robinson 2008). However, the comparison of Arctic rate estimates in Table 3 and published relationships linking respiration

to PP across systems (del Giorgio *et al*., 1997) suggest that bulk C availability and quality obviously plays a role in determining respiration. Therefore it is difficult to imagine that respiration was not in some way related to C availability in this time-series. The negative relationship of CR with NO_3^- concentration most likely reflects the higher rates of C availability associated with NO_3^- draw down by phytoplankton and not a specific link to NO_3^- itself. Nonetheless, it is possible that there was a combined effect, an inter-play between post-bloom OC quality, reduced lability and inorganic nutrient availability, that resulted in higher CR, with concomitant lower BP.

BR was derived empirically (Robinson 2008) and was over the course of the time series estimated to be from 41-49% of CR. Therefore patterns in estimated BR mimicked patterns in CR quite closely. Given the seasonal variability of BP and the more constrained BR estimates, the pattern in BGE and its predictability were predominantly driven by changes in BP. BGE was low in the Amundsen Gulf, averaging 4.6% throughout the entire period with very low winter rates of slightly less than 1%, a maximum of 18.6%, and an average late spring-summer rate at 6.6%. Despite the conversions made in this study to derive seasonal BGE estimates, our values are similar to those reported for other Arctic regions (Kirchman *et al.,* 2009a, Kritzberg *et al.,* 2010). These lower BGE estimates support the idea that we are underestimating BR from BP when extrapolating it using an average BGE of 15% or greater in polar oceans (Garneau *et al.,* 2008, Robinson *et al.,* 1999). It has been suggested that low BGEs reflect very hostile environments and that perhaps the average oligotrophic oceanic BGE hovers more around 8% rather than the more commonly referred to 15% (Carlson *et al.,* 2007). Results from Arctic regions certainly support this suggestion.

Cell-specific rates of BP and BR give additional insight into the dynamics of microbial metabolism and their controlling factors. Although BP and BR explained an equal amount of variability in BA, BP_{sp} rates were significantly higher during the summer period, whereas the average winter and summer BR_{sp} rates were not significantly different. Thus, unlike BP, the peak in BR is likely a function of greater abundance rather than increased rates at the cellular level. Looking at temperature corrected cell-specific BR rates, Lopez-Urutia and Moran (2007) observed a strong positive relationship between Chl *a* and both BP_{sp} and BGE, while no relationship was observed with BR_{sp}, suggesting a stronger impact of substrate limitation on BP_{sp} and its predominant role in driving BGE, similarly to our study. This could explain the

observed uncoupling of BP and respiration peaks: BP would increase rapidly with substrate availability, whereas the peak in respiration would occur later when microbial abundance was high but substrate availability low during post bloom conditions. Our findings were supported independently using a dissolved inorganic carbon (DIC) mass balance approach in 2008 in the Amundsen Gulf, where a one-month lag between peak PP and peak respiration was also observed (Shadwick *et al.,* 2011).

3.4.2 Implications for C cycling in Amundsen Gulf

The high respiration rates measured throughout most of the year in this study, suggest high microbial C demand in the Amundsen Gulf. Our spring-summer averages of CR integrated over the top 80 m of the water column were equal to 225 (±93) g C m^{-2}. If we consider the GPP of 52.4 g C m^{-2} for this same period, estimated by dividing NO_3-based new PP by the average *f*-ratio during this study (Forest *et al.*, 2011), the system is in a significant C deficit. Large C deficits of 121 (Kirchman *et al.*, 2009a) to 737 mg C m^{-2} d^{-1} (Cota *et al.,* 1996) have also been observed in the Chukchi Sea, where measured respiration was much higher than ^{14}C estimates of PP during the spring and summer. However ^{14}C estimates are known to underestimate GPP (Howarth and Michaels 2000) and may explain some of the deficit observed in these studies. Several possibilities could explain this missing C: 1) respiration is overestimated, 2) allochthonous sources of OC are critical to supply microbial demands, 3) GPP is underestimated and/or 4) the system is heavily reliant on C recycling.

There are a number of reasons why CR could be overestimated. Accurate measures of respiration are difficult to acquire and subject to methodological limitations (Robinson and Williams, 2005), particularly in cold environments. Relatively long incubations could artificially increase CR rates, in part due to bottle effects. The latter however is minimized when rates are linear over the course of the incubation (Robinson, 2008), as was the case for the measurements made in this study. One of the more important constraints of our reported CR rates is the choice of the Q_{10} conversion. Several studies have shown that when cold-water communities are subject to temperature deviations, the Q_{10} is often higher than the

Table 3. Compilation of volumetric microbial metabolic rates and bacterial growth efficiencies from published reports from the Arctic Ocean.

Region	Depths	Period	BA (10^5 cells ml^{-1})		CR (μg C L^{-1} d^{-1})		BR (μg C L^{-1} d^{-1})		BP (μg C L^{-1} d^{-1})		BGE (%)		Source
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Amundsen Gulf	Whole WC	Annual	2.73	0.72-12.3	15.5	3.8-44.2	6.74[b]	1.84-18.1[b]	0.146	0-2.68	4.59	0.20-18.6	This study
Canadian Arctic		November-April	1.63	0.77-2.48	11.1	3.8-22.5	4.94[b]	1.84-9.64[b]	0.053	0-0.242	1.82	0.20-5.63	
		May-July	3.90	0.72-12.3	18.7	6.7-44.2	8.05[b]	3.14-18.1[b]	0.358	0.004-2.68	6.60	0.44-18.6	
Chukchi Sea	Photic Layer	Spring-Summer	7.4	2.7-10							6.9	0.6-44	Kirchman et al. 2009
Western Arctic		Summer 2004			42.6	0-191.2	16.8	0.23-75.8					
Central Arctic	Upper 240m	Winter	1.5	0.72-2.9	2.9	0-15.9	1.4[b]	0-7.0[b]	0.012	0.005-0.06	0.85[d]		Sherr and Sherr 2003
		Summer	2.2	0.72-6.7	9.3	3-28.3	4.2[b]	1.5-12[b]	0.130	0.043-0.36	3.0[d]		BA in Sherr et al. 2003
Franklin Bay	Whole WC	Autumn	1.2						0.045[c]				Garneau et al. 2008
Canadian Arctic		Winter	3.1						0.016[c]				
		Spring	2.0						0.015[c]				
		Summer	6.8						0.274[c]				
Fram Strait	Photic Layer	All			34.1	0.096-280	14.2[b]	0.06-101[b]					Vaquer-Sunyer et al. (submitted)
		Spring			9.3		4.2[b]						
		Summer			36.3		15[b]						
		Winter			8.1		3.7[b]						
Chukchi Sea	Photic Layer	Summer 2002			14.3		6.32						Cottrell et al. 2006
Western Arctic		Spring 2004			39.7		16.3						
		Summer 2004			26.1		11.1						
Kara Sea	Whole WC	August-Sept.	3.5	2.3-4.7			9.5	3.3-21	2.24	0.67-6.9	16.4	10-31	Meon and Amon 2004

[a]Potential bacterial respiration, BR; bacterial production; BP, bacterial abundance, BA; community respiration, CR; potential bacterial growth efficiency, BGE.
[b]Values based on the equation $BR=0.45CR^{0.93}$ [*Robinson*, 2008].
[c]Values presented are medians.
[d]Estimated using BP and BR means from current Table.
WC= water-column
Note: When necessary, O_2 respiration rates were converted to C units using a respiratory quotient of 0.8 for consistency with the present study.

canonical value of 2 (Apple *et al.,* 2006, Kritzberg *et al.,* 2010, Martinez 1996, Vaquer-Sunyer *et al.,* 2010, Yager and Deming 1999). This issue is believed to be especially significant when these deviations occur at low *in situ* temperatures (Kirchman *et al.,* 2009b, Pomeroy and Wiebe 2001), as in the present study. The average Q_{10}s for CR from these studies was 4, with bacterial Q_{10}'s averaging 6, however some extreme values reported for the Arctic are > 15. In our case, most of our incubations were higher than *in situ* temperature, meaning that the use of a canonical Q_{10} of 2 would provide an even higher respiration estimate. Therefore we opted for the more realistic and ultimately more conservative Q_{10} of 4 for our Arctic samples. However, if we double the average cold temperature Q_{10} of 4 to 8 in order to present a more conservative estimate of respiration, the value would be 144 gC m^{-2} and would still exceed PP. Nevertheless, our respiration estimates are on par with all other reports published for the Arctic (Table 3) regardless of the methods used, supporting a generally high and consistent respiratory demand in the Arctic. There is obviously a great need to better elucidate the impact of warming on the Q_{10} of metabolic rates in the Arctic given the rapid changes occurring in the system as a function of climate forcing (Wassmann 2011), as warming may result in a greater than anticipated change in system C demand.

Allochthonous inputs of OC could help compensate potential C deficits in Arctic Ocean. This system receives relatively high terrestrial inputs from rivers (Dittmar and Kattner 2003, Peterson *et al.,* 2002) and is subject to important coastal and seabed erosion (Carmack and MacDonald 2002, McGuire *et al.,* 2009, O'Brien *et al.,* 2006), all of which are expected to increase C loading to the Arctic with regional climate warming. Atmospheric deposition may also be a relatively important external source of OC to the system (Macdonald *et al.,* 2004). With turnover times on the scale of years, terrestrial C is usually considered recalcitrant (Hansell *et al.,* 2004), and is thought to be a minor source of OC available to organisms relative to C derived from primary production, at least seasonally for productive surface waters. Fresher material loading to the system during periods of peak run-off may provide some regional relief from C deficits, either through direct loading or from advection to sites further from shore. The extent and significance of the biological processing of terrestrial C in the Arctic Ocean remains poorly characterized (Benner *et al.,* 2004) and merits further consideration.

Another explanation for the discrepancy between CR and GPP is that GPP is underestimated. However, while incubation-based estimates of GPP are subject to bottle effects

(Quay *et al.,* 2010), this does not apply to the non-incubation based estimate from Forest *et al.* (2011), where GPP is back-calculated by dividing NO_3-based new PP by the average *f*-ratio. In the aforementioned study, the background DOC pool for the upper 100 m remained high and relatively constant (~80-95 mg C m^{-2}) from February to August 2008, with a 133 mg C m^{-2} peak in July that rapidly declined to background averages (Forest *et al.*, 2011). The latter suggests a rapid production and consumption of ~50 mg C m^{-2} on a time scale of days and represents an important pulse in C to the system. Such pulses could be fuelled by short local upwelling events which re-supply nitrate in the euphotic layer. These events are not always accounted for in nitrate drawdown inventories, and could underestimate GPP when derived from new PP and f-ratios. These short-lived events could help fill the gap of observed C deficits (Karl *et al.,* 2003). Furthermore pulse events may become more important in coastal areas, where deep-water upwelling as a function of climate forcing in a rapidly changing Arctic, may result in overall higher productivity (Tremblay *et al.,* 2011). Alternative C pathways, such as dark CO_2 fixation by heterotrophic (Alonso-Saez *et al.,* 2010) or autotrophic prokaryotes may also be important autochthonous C inputs to the Arctic, but remain poorly constrained. These may be particularly critical in supplying C during the winter months and at depth.

In a high-resolution study of the 2007-2008 DIC fluxes in Amundsen Gulf, Shadwick *et al.* (2011) conclude that the region is globally heterotrophic with an annual net-autotrophic surface layer with brief periods of net-heterotrophy occurring in winter. DIC mass balance models are based on the deviation of measured from expected DIC values as a function of physical properties and represent the endpoint results of integrated biological processes. DIC-based estimates however use net community production (NCP) in their models and these values will be much lower than GPP under conditions of high remineralization, where part of the DIC used by primary producers originates from *in situ* respiration (Mathis *et al.,* 2009). In fact if we apply an *f*-ratio more representative of system subject to elevated rates of recycling, such as 0.2 as compared to the 0.6 estimated by Forest *et al.* [2011] at the peak of the productive season, estimated GPP would be around 166 g C m^{-2} thus closing the gap with our most conservative respiration estimates. Annual time series of *f*-ratios for the Arctic are rare, but values ranging from 0.05 to 0.38 have been observed in the Chukchi Sea (Cota *et al.,* 1996). The variability in the *f*-ratio is likely to influence final estimates as these have been shown to rapidly decrease to

values closer to 0.1 - 0.4 upon nitrate depletion in the euphotic layer in the North Water Polynya (Tremblay *et al.,* 2006a). Future investigations are required to better constrain *f*-ratios and their variability in the Arctic Ocean to derive improved estimates of GPP. Furthermore recycling from the food web seems to be of prime importance in the Arctic (Forest et al. 2011) and the potential for active nitrification in the photic layer (Christman *et al.,* 2011, Yool *et al.,* 2007) may mask the true contribution of regenerated PP to GPP.

Interestingly there are multiple lines of evidence to support that this system is strongly supported by internal recycling processes. First, there is consistently low export of OC from the surface to the deep layers in this region (Forest *et al.,* 2010, Sallon *et al.,* 2011) where high extracellular enzyme activity has been measured on sinking particles (Kellogg *et al.,* 2011). Secondly there is sustained microbial activity observed throughout the winter (this study; Alonso-Saez *et al.*, 2008, Garneau *et al.*, 2008), along with active grazer dependence on recycled materials (Sampei *et al.,* 2009). While we acknowledge the shortcomings of rate-based measurements, our study and others seem to support regional annual heterotrophy in the Amundsen Gulf (Garneau *et al.*, 2008, Shadwick *et al.*, 2011). We suggest that the most plausible explanation to reconcile some of the observed discrepancies is that the system is heavily dependent on recycling of C and nutrients through microbes and the rest of the food web.

3.5 Conclusion

This study presents a unique time series of microbial C dynamics in the Arctic Ocean where we were able to measure relatively constant rates of respiration throughout the winter, with slight increases in rates during the spring and summer. Although subject to methodological constraints, the measured high rates of respiration and estimated low BGEs in the Amundsen Gulf are similar to other published reports for the Arctic. Our findings confirm that a large amount of OC is being channeled towards respiration in the Amundsen Gulf, and would be indicative of a high level of recycling within the system. How warming influences the metabolic balance of the Arctic remains an exciting line of inquiry. Although climate warming may result in increased productivity (Tremblay *et al.*, 2011), some studies suggest relatively greater respiratory losses (Kritzberg *et al.,* 2010, Vaquer-Sunyer *et al.,* 2010, Wohlers *et al.,* 2009), altering system C demand and dynamics. Given the apparently large amounts of C

respired in the Arctic, there is an urgent need to refine the parameterization of metabolic estimates as a function of warming given the impending ecosystem scale alterations anticipated in future climate.

3.6 Acknowledgements

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3.7 Supplementary information

Due to logistical and space limitations during sampling, we were unable to conduct all of our respiration measurements at *in situ* temperature. Corrections factors were therefore applied to measured respiration rates to account for the deviations from *in situ* temperature during incubations. Samples were often incubated at a temperature of between 2 and 4 °C, which was typically higher then temperature *in situ* (Figure 1). Therefore applying the correction would lower the respiration rate we measured (Table 1). Table 1 reports all raw CR measurements along with Q_{10}-corrected values based on a Q_{10} of 2 and 4. This table illustrates how, even when using a higher correction factor (i.e.Q_{10}=4) respiration rates remain relatively high in winter. Figure 1 shows the impact of the Q_{10} correction of 4 on CR in relation to *in-situ* temperature. We acknowledge that ideally incubations should have been kept at or closer to *in situ* temperature. Although logistically challenging, this supplementary information highlights the need that future studies looking at respiration rates in the Arctic should aspire to do this.

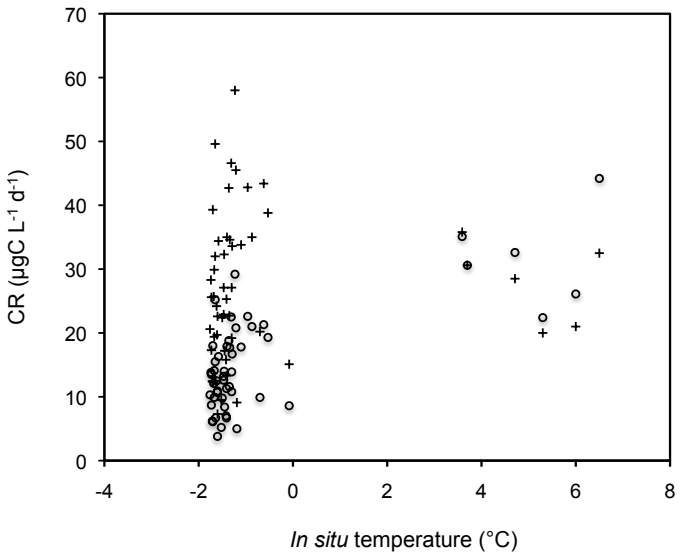
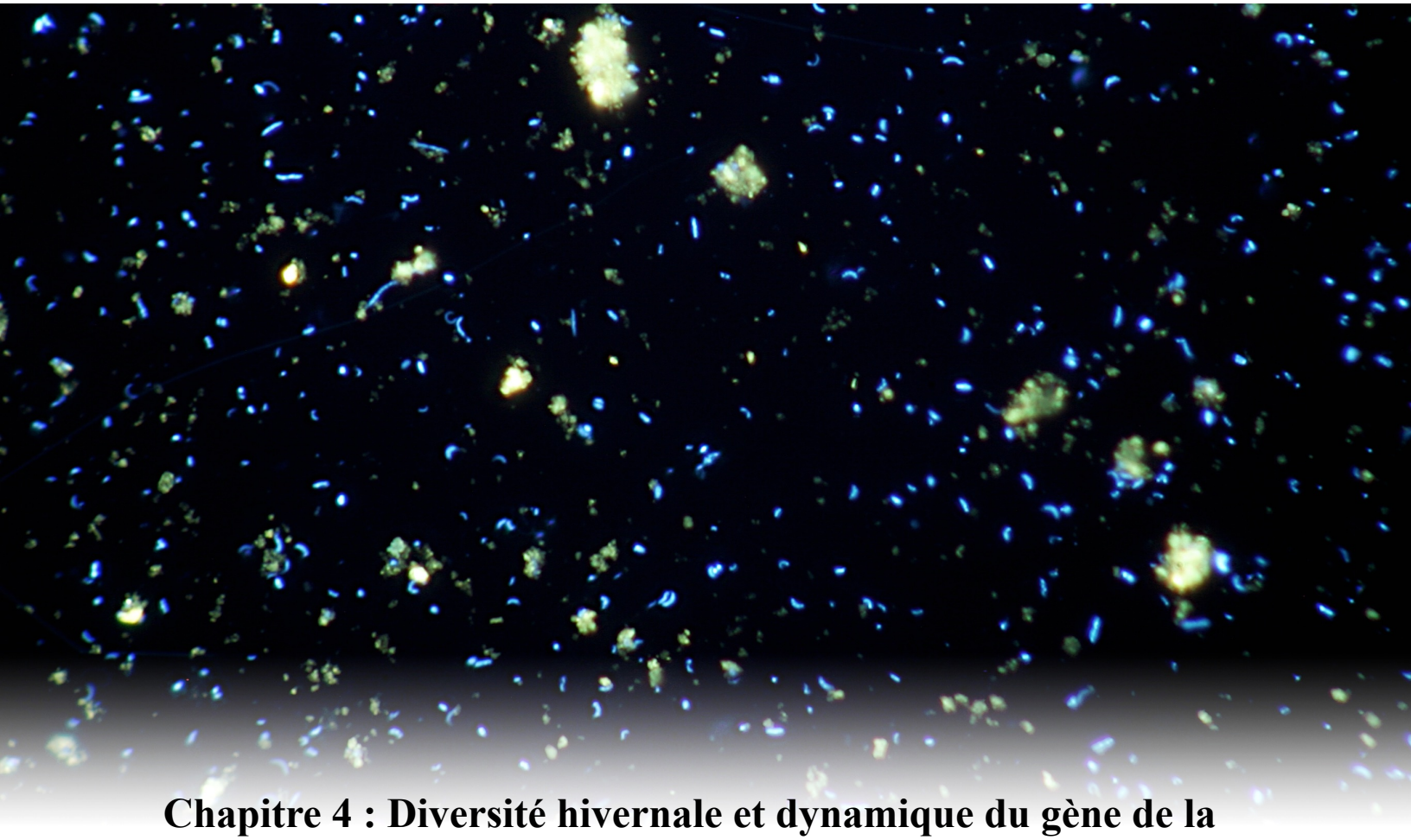


Figure 1. Comparison of the uncorrected (crosses) and CR corrected with a Q_{10} of 4 (open circles) as per the manuscript as a function of *in-situ* temperature.

Table S1. Comparison of uncorrected and corrected CR estimates using Q_{10} values of 2 and 4, with *in-situ* and incubation temperature, geographical localization, and depth sampled.

Date dd-mm-yyyy	Latitude N dec. degrees	Longitude W dec. degrees	Depth m	$T°_{in\ situ}$ °C	$T°_{incub}$ °C	ΔT° °C	CR_{raw} µgC L^{-1} d^{-1}	$CR_{Q10=2}$ µgC L^{-1} d^{-1}	$CR_{Q10=4}$ µgC L^{-1} d^{-1}
19-11-2007	70.6217	123.0142	10	-1.65	3.24	4.9	49.6	35.3	25.2
19-11-2007	70.6217	123.0142	82	-1.76	3.24	5.0	20.6	14.6	10.3
2-12-2007	71.7322	125.5633	10	-1.62	3.16	4.8	24.2	17.4	12.5
2-12-2007	71.7322	125.5633	60	-1.19	3.16	4.3	9.1	6.7	5.0
10-12-2007	71.2662	125.2540	10	-1.65	3.58	5.2	32.0	22.3	15.5
17-12-2007	71.4817	126.3806	10	-1.61	2.80	4.4	19.7	14.5	10.7
17-12-2007	71.4817	126.3806	100	-1.60	3.11	4.7	7.3	5.3	3.8
8-1-2008	71.5264	125.5961	10	-1.64	2.82	4.5	12.5	9.2	6.7
25-2-2008	70.9407	123.9226	12	-1.73	2.89	4.6	25.6	18.6	13.5
25-2-2008	70.9407	123.9226	60	-1.52	2.89	4.4	9.5	7.0	5.2
17-3-2008	70.9080	123.4770	10	-1.68	3.76	5.4	25.7	17.6	12.1
17-3-2008	70.9080	123.4770	50	-1.58	3.80	5.4	34.4	23.7	16.3
17-3-2008	70.9080	123.4770	100	-1.45	3.74	5.2	17.2	12.0	8.4
26-3-2008	71.0640	121.7867	10	-1.70	3.94	5.6	39.3	26.6	18.0
26-3-2008	71.0640	121.7867	60	-1.31	3.94	5.2	46.6	32.4	22.5
26-3-2008	71.0640	121.7867	160	-0.08	3.94	4.0	15.1	11.4	8.6
6-4-2008	71.1895	121.0867	10	-1.73	3.22	5.0	17.3	12.3	8.7
6-4-2008	71.1895	121.0867	60	-1.67	3.22	4.9	19.4	13.8	9.9
12-4-2008	71.2450	124.6117	10	-1.74	3.44	5.2	28.3	19.7	13.8
12-4-2008	71.2450	124.6117	90	-1.40	3.44	4.8	35.0	25.0	17.9
16-4-2008	70.7900	122.3400	10	-1.71	3.37	5.1	12.4	8.7	6.2
16-4-2008	70.7900	122.3400	30	-1.70	3.37	5.1	12.4	8.7	6.1
19-5-2008	70.6604	122.8798	12	-1.34	3.50	4.8	34.6	24.8	17.7
19-5-2008	70.6604	122.8798	30	-1.35	3.50	4.9	22.7	16.2	11.6
19-5-2008	70.6604	122.8798	50	-1.41	3.50	4.9	13.3	9.5	6.7
23-5-2008	72.6499	127.3969	12	-0.70	4.41	5.1	20.2	14.2	9.9
23-5-2008	72.6499	127.3969	50	-1.41	4.41	5.8	25.3	16.9	11.3
23-5-2008	72.6499	127.3969	60	-1.42	4.41	5.8	15.8	10.5	7.0
2-6-2008	69.8599	123.7519	10	-0.87	2.83	3.7	35.0	27.1	21.0
2-6-2008	69.8599	123.7519	20	-1.47	2.83	4.3	22.9	17.0	12.6
2-6-2008	69.8599	123.7519	40	-1.30	2.83	4.1	19.2	14.4	10.8
9-6-2008	69.8267	123.6308	10	-0.96	3.63	4.6	42.8	31.1	22.6
9-6-2008	69.8267	123.6308	30	-1.60	3.63	5.2	22.6	15.7	10.9
14-6-2008	69.9795	125.8699	0	-1.36	4.57	5.9	42.7	28.3	18.8
14-6-2008	69.9795	125.8699	20	-1.46	4.57	6.0	32.3	21.3	14.0
15-6-2008	69.9563	125.8750	12	-0.53	4.51	5.0	38.8	27.4	19.3
15-6-2008	69.9563	125.8750	17	-0.62	4.51	5.1	43.4	30.4	21.3
28-6-2008	71.0648	126.0468	2.5	3.70	3.71	0.0	30.6	30.6	30.6
28-6-2008	71.0648	126.0468	35	-1.23	3.71	4.9	58.0	41.2	29.2

Date dd-mm-yyyy	Latitude N dec. degrees	Longitude W dec. degrees	Depth m	$T°_{in\ situ}$ °C	$T°_{incub}$ °C	ΔT° °C	CR_{raw} µgC L^{-1} d^{-1}	$CR_{Q10=2}$ µgC L^{-1} d^{-1}	$CR_{Q10=4}$ µgC L^{-1} d^{-1}
28-6-2008	71.0648	126.0468	71	-1.47	3.71	5.2	27.1	18.9	13.2
30-6-2008	71.4725	133.5542	2.7	4.71	3.74	-1.0	28.5	30.5	32.6
30-6-2008	71.4725	133.5542	71	-1.29	3.74	5.0	33.6	23.7	16.7
30-6-2008	70.1779	133.5542	2.7	3.59	3.74	0.2	35.8	35.5	35.1
30-6-2008	70.1779	133.5542	35	-1.67	3.74	5.4	29.9	20.5	14.1
2-7-2008	71.0789	133.7996	4.5	5.30	4.49	-0.8	20.0	21.1	22.4
2-7-2008	71.0789	133.7996	65	-1.50	4.49	6.0	22.4	14.8	9.8
4-7-2008	72.6588	128.3601	2.8	6.00	4.44	-1.6	21.0	23.5	26.1
4-7-2008	72.6588	128.3601	52	-1.21	4.44	5.6	45.5	30.8	20.8
10-7-2008	71.2884	127.7568	2.6	6.50	4.28	-2.2	32.5	37.9	44.2
10-7-2008	71.2884	127.7568	58	-1.10	3.53	4.6	33.8	24.5	17.8
10-7-2008	71.2884	127.7568	70	-1.30	3.53	4.8	27.1	19.4	13.9



Chapitre 4 : Diversité hivernale et dynamique du gène de la protéorhodopsine dans un océan polaire

Photo: Bactéries (en bleu) et matière organique (vert et jaune) de l'Océan Arctique, visualisées par épifluorescence.

Winter diversity and expression of proteorhodopsin genes in a Polar Ocean

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Running title : Proteorhodopsin dynamics in a polar ocean

Keywords : Carbon cycle / diversity / expression / polar / proteorhodopsin / seasonal dynamics

Subject category : Microbial ecology and functional diversity of natural habitats

Submitted to the ISME journal

Abstract

Mixotrophy is a valuable functional trait used by microbes when environmental conditions vary broadly or resources are limited. In the sunlit waters of the ocean, photoheterotrophy, a form of mixotrophy, is often mediated by proteorhodopsin (PR), a seven helices transmembrane protein binding the retinal chromophore. Together, they allow bacteria to capture photic energy for sensory and proton gradient formation cell functions. The seasonal occurrence and diversity of the gene coding for PR in cold oligotrophic polar oceans is not known and PR expression has not yet been reported. Here we show that PR is widely distributed among bacterial taxa, and that PR expression decreased markedly during the winter months in the Arctic Ocean. Gammaproteobacteria-like PR sequences were always dominant. However, within the second most common affiliation, there was a transition from Flavobacteria-like PR in early winter to Alphaproteobacteria-like PR in late winter. The phylogenetic shifts followed carbon dynamics, where patterns in expression were consistent with community succession, as identified by DNA community fingerprinting. Although genes for PR were always present, the trend in decreasing transcripts from January to February suggested reduced functional utility of PR during winter. Under winter darkness, sustained expression suggests that PR may continue to be useful for non-ATP forming functions, such as environmental sensing or small solute transport. The persistence of PR expression in winter among some bacterial groups may offer a competitive advantage, where its multi-functionality enhances microbial survival under harsh polar conditions.

4.1 Introduction

Mixotrophic strategies are important, often overlooked components of the microbial ecology in aquatic ecosystems (Eiler 2006). Bacterial photoheterotrophy, a form of mixotrophy, combines the use of organic substrates with light energy and is increasingly thought to be a common strategy among bacteria in marine surface waters. The broad distribution of the proteorhodopsin (PR) gene suggests that PR may be a particularly widespread mechanism for facilitating photoheterotrophy (Béjà et al., 2000, Béjà et al., 2001). PR, together with its membrane-bound retinal chromophore, mediates light-activated proton pumping for both energy production and sensory function in bacteria. Rhodopsins have been associated with light-enhanced growth and survival for some cultivated strains (Gómez-Consarnau et al., 2007, Gómez-Consarnau et al., 2010), but not for others (Giovannoni et al., 2005, Riedel and Tomasch 2010, Riedel et al., 2013, Stingl et al., 2007) suggesting both photoheterotrophy and broader functional roles of this gene (Furhman et al., 2008). Although the characterization of the latter remains elusive, support for the diversity of PR functions has been found in previous studies (Bamman et al., 2014, Mongodin et al., 2005, Riedel et al., 2013, Yoshizawa et al., 2014).

PR has been identified in many oceans and seas (Béjà et al., 2000, Cottrell and Kirchman 2009, Riedel and Tomasch 2010) and is phylogenetically diverse (Rusch et al., 2007). Microbial rhodopsins are highly abundant in the photic biosphere. A metagenomic survey found them to be present on average in 48% of cells, and in some cases in all cells from both marine and terrestrial samples (Finkel et al., 2013). Despite the pervasive presence of PR genes in nature, there are limited data on PR expression. Indeed, only a few studies to date report PR transcripts from natural communities, with most reports gleaned from short term, localized metatranscriptomic studies (Frías-López et al., 2008, Poretsky et al., 2009). Patterns in PR expression would be expected to vary seasonally and co-vary as a function of light availability, however such patterns have yet to be explored. Although PR genes have been reported during both light-replete summer and light-limited winter at an Arctic coastal site (Kirchman and Cottrel 2009), detailed long-term seasonal dynamics of PR's presence or expression in Polar oceans have yet to be described.

Bacteria in the upper Arctic Ocean remain active throughout the year, despite the lack of light, sub-zero temperatures, and low substrate concentrations in winter (Garneau et al., 2008,

Kirchman et al., 2009, Nguyen et al., 2012). However, mechanisms for such persistence are unclear. These bacterial communities have some of the lowest growth efficiencies (BGEs) reported in marine environments, with mean seasonal values ranging from 2 to 7% (Kirchman et al., 2009, Nguyen et al., 2012) where lowest values are observed in winter. Such low BGEs suggest that most of the available energy is diverted towards maintenance rather than active growth, making PR-generated ATP a potentially precious commodity in the carbon economy of Polar Oceans. Alternatively, PR may contribute to bacterial survival in other ways, such as enhanced motility, sensing and active transport of small molecules (Fuhrman et al., 2008). Indeed, PR could provide multiple functions for the same or different organisms over time and space, potentially providing bacteria possessing the gene an advantage over those that do not under varied conditions.

We tracked the seasonal prevalence and phylogenetic diversity of a marker for PR over eight consecutive months, as part of the International Polar Year (IPY) Circumpolar Flaw Lead (CFL) study (Barber et al., 2010). Our core objectives were to 1) follow seasonal changes in the PR signal from November to July through multiple-primer polymerase chain reaction (PCR) amplifications, 2) characterize PR expression patterns using reverse transcribed PCRs, and 3) look at the variations in winter diversity of bacteria possessing the PR-gene via cloning and sequencing. We expected PR distribution and expression to follow seasonal cycles, where the gene would gradually disappear with decreased sunlight during winter and reappear in the spring with greatest prevalence under 24 h daylight during the Arctic summer. Unexpectedly, PR was recovered throughout the Arctic winter, along with evidence for its expression during this time suggesting other important functional roles of this gene that are not dependent on light availability.

4.2 Material and methods

4.2.1 Study site

The study was carried out onboard the CCGS Amundsen in the Amundsen Gulf of the Southeastern Beaufort Sea. Weekly samples were collected from November 2007 to June 2008. Detailed physical and chemical conditions during the IPY-CFL study are reported in (Barber *et al.,* 2010). Overall, 16 surface seawater samples were selected for this study (Figure S1).

4.2.2 Sample collection and DNA preparation

Water samples were collected using a rosette system equipped with twenty-four 12-L Niskin-type bottles. Three to six L of water were sequentially filtered through 53-µm nylon mesh, 3-µm pore size polycarbonate (PC) filter (Millipore), with samples for DNA collected in a 0.2 µm filter cartridge (Sterivex, Millipore) and preserved in buffer (40mM EDTA; 50mM Tris pH=8.3; 0,75 M Sucrose). Samples for RNA were collected onto 47-mm 0.22 µm pore size PC membrane filters (Millipore), and preserved in 2 ml cryovials with 600 µl of RLT buffer (Qiagen) and 1% beta-mercaptoethanol (Sigma–Aldrich). Filters were kept at -80 °C and were extracted within two years after collection. Salt-extraction of microbial DNA was adapted from Aljanabi and Martínez (1997) and included steps using lysozyme, proteinase K, and sodium dodecyl sulfate (Terrado *et al*., 2011). RNA was extracted as in Church *et al.* (2005), using an RNAeasy kit (Qiagen) and converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with random primers and an RNase inhibitor. Extraction of nucleic acids yielded concentrations ranging from 5.3 to 291 ng μl^{-1} in DNA samples that were measured using a NanoDrop© 1000 spectrophotometer (Thermo Scientific). The cDNA concentrations ranged from 0.28 to 2.1 ng μl^{-1} and were measured using the PicoGreen© (Molecular Probes Inc.) fluorometric method. As RNA was not quantified prior to reverse-transcription, we assume cDNA concentrations were representative of the original RNA concentrations in samples.

4.2.3 PR Primers and PCR amplification

Degenerate PR primers were modified from Atamna-Ismaeel *et al*. (2008) and Koh *et al*. (2010), with an expected PCR product size of ~350bp. An additional reverse primer (PR-R4) was created after *in silico* testing with the Fuzznuc web application (http://emboss.bioinformatics.nl/cgi-bin/emboss/fuzznuc). Consensus sequences were added to the 5' ends of primers to increase amplification efficiency (Bodaker *et al.,* 2012). Six primer pairs from Koh *et al*. (2010) were used in combination with a seventh pair using the additional reverse primer (SI Table 1). It should be noted that sequences representative of the actinorhodopsin-like LG1 and LG2 groups (Sharma *et al.*, 2008, 2009) present a TWXXYP region at the 3' end and would not be amplified by our set of reverse primers that are based on GWXXYP (Atamna-Ismaeel *et al.*, 2008) and SWXFY regions. PCRs were performed with

the hot start Platinum Taq DNA Polymerase (Invitrogen). Optimal PCR amplification followed: 1 cycle of 95°C for 3 min, 40 cycles of 94°C for 30 s, 48.5°C for 30 s and 72°C for 1 min, and a final cycle of 72°C for 10 min. Target load of template for the PCR reactions was 10 ng for DNA. Since cDNA concentrations were low we did not achieve that target load and a 1.5 ng cDNA template per reaction was used. An environmental sample from the Blanes Bay Microbial Observatory time-series, where PR presence was confirmed by DNA sequencing was used as a positive control. Nanopure water was used as a negative control. Particular attention was given to ensure conditions were kept constant between all reactions throughout the amplification procedure. Although, precise information on the efficiency of each primer pair relative to one another is unavailable, we assume that within each primer pair, all other things being equal, the differences in signal intensity can be reasonably attributed to differences in initial target DNA and cDNA, which is the underlying assumption of fingerprinting techniques as well as high throughput sequencing studies (Díez *et al.,* 2004, Gilbert *et al*., 2009, Schauer *et al.,* 2003)

4.2.4 Clone libraries

PCR products were cloned using the Agilent Technologies StrataClone PCR cloning kit using competent cells of *Escherichia coli* (Agilent Technologies). Colonies were screened by PCR to verify clonal insert size and approximately 500 clones were retained for standard Sanger sequencing (Genoscreen, France) using a 3730XL DNA Analyzer (Applied Biosystems) to confirm proteorhodopsin amplification. Specific PR primers (Table S1) were used during sequencing rather than the adjacent T7 or other plasmid sequencing primers to minimize sequencing non-PR clones. This restriction however, resulted in PR fragments shorter (150-279bp, for an approximate 20 to 37% coverage of the gene) than the expected target after trimming primers and poor 5' ends. After manual removal of low quality sequences (those with multiple ambiguous bases and Ns), 188 PR-clones were kept for subsequent analysis following a basic local alignment search (BLAST) against the NCBI nr database. Nucleotide sequences were clustered into operational taxonomic units (OTUs) at a 6% dissimilarity threshold (Riedel and Tomasch 2010) using MOTHUR (Schloss 2009).

PR gene sequences were deposited in GenBank under accession numbers KJ937475-KJ937662.

4.2.5 PR phylogeny

The amino acid sequences of PR clones were manually curated and aligned against a reference PR alignment with the *–seed* function of the MAFFT algorithm (Katoh and Toh 2010). Cleaning and alignment of sequences against known PR sequences, showed our fragments were relatively short and reliable reads were only observed from positions 109-111. The fragments included site A178 involved in conformational changes in the E and F helices but they did not include regions coding for spectral tuning (L105), electron donor (E108) or electron acceptor (D97) that were on the low quality start of the sequences. With the exception of 5 sequences were the E108 position was present and indicated the proton pumping conformation, this precluded us from carrying further analysis on the aforementioned sites. Bootstrap resampling and maximum likelihood-based (ML) phylogenetic analyses were carried out using RAxML (Stamatakis 2006, Stamatakis *et al.,* 2008) and based on a GAMMAWAGF evolutionary model, selected using the ProtTest web application (http://darwin.uvigo.es/software/prottest2_server.html). Tree visualization and design was done using the Interactive Tree of Life (Letunic and Bork 2011, www.itol.org).

4.2.6 Environmental variables

Temperature and salinity profiles were obtained on the downward casts of the rosette equipped with a conductivity temperature depth (CTD) profiler (SeaBird 911+ CTD) with a sensor for photosynthetically active radiation (PAR). Daily above-ice irradiance at noon (kindly provided by Dr. T. Papakyriakou) was used as an indicator of seasonal trends in irradiance, rather than the underwater PAR data, as water sampling was carried out at varying time of day depending on ship operations and movements. Nutrient samples were collected directly from the Niskin-like bottles and concentrations of nitrate+nitrite (NO3-+NO2-) and nitrite (NO2-) were determined on board with a Bran and Luebbe Autoanalyzer 3 using routine colorimetric methods (Grasshoff et al., 1999). Since nitrite levels were low, NO3-+NO2- is hereafter referred to as NO3-. Samples for chlorophyll a (Chl a) were filtered onto 25 mm diameter Whatman GF/F filters (nominal pore size 0.7 mm). Filters were extracted onboard in 90% acetone over 24 h at 5°C in the dark. Chl a fluorescence was measured using a Turner Designs fluorometer model 10-AU before and after acidification (Parsons et al., 1984). Chl a concentrations were then calculated using equations of Holm-Hansen et al. (1965).

4.2.7 Bacterial Abundance and Production

Water samples for total bacterial abundance were preserved for 1 h in (5% v/v) formaldehyde and filtered onto 0.2 µm pore diameter black polycarbonate filters after staining with 4'6'-Diamidino-2-phenylindole dihydrochloride (DAPI). Concentrations were determined using epifluorescence microscopy (Porter and Feig 1980) and counts include both Archaea and Bacteria, but are referred to as bacteria for simplicity. Bacterial production was measured using 3H-leucine incorporation (Smith and Azam 1992) where rates of leucine incorporation were corrected for radioactivity adsorption using TCA killed controls. Detailed procedures on cell counts and bacterial production are given in Nguyen et al. (2012).

4.2.8 Denaturing gradient gel electrophoresis

Bacterial community changes over the seasons were first evaluated using denaturing gradient gel electrophoresis (DGGE) fingerprinting (Schauer et al., 2003). Briefly, DGGE was carried out on a DGGE 2000 system (CBS scientific) where partial 16S rRNA genes were amplified using the bacterial specific forward primer GC358F with a GC clamp, and the universal reverse primer 907RM. About 800 ng of the PCR product was loaded onto a 6% acrylamide gel containing a denaturing gradient of 40 to 80%, where 100% of the denaturing agent contained 7 M urea and 40% deionised formamide. Gels were run at 100V for 17h at 60°C. Following staining with Sybr Gold™ (Invitrogen) and image capture, a matrix of the relative contribution of bands to the total intensity of each lane was built using the Quantity One software (Bio-Rad). From this matrix, a distance matrix was calculated using the Hellinger dissimilarity index. This matrix was used for UPGMA clustering. Multi-scale bootstrap analyses were done using the pvclust package in the R statistical package in order to test the stability of clusters.

4.3 Results

4.3.1 Environmental and bacterial dynamics

Strong seasonal differences were evident over the study from November 2007 to July 2008 (Fig. 1). Temperature remained near the freezing point of seawater (-1.7 °C) for most of the study and increased after ice-melt in the late spring (Fig. 1A). Total surface solar

irradiance and PAR (Fig. 1B) sharply decreased in November and rapidly increased in February, reaching maximum values in May and June. Phytoplankton biomass, estimated from Chl *a* concentrations (Fig. 1C), roughly followed irradiance, increasing from February to April (slope=0.01, $p<0.0001$). Over May and June Chl *a* levels were on average higher but with a large degree of scatter over the sampling region; values declined slightly in the second half of June (slope=-0.06, $p=0.014$). NO_3^- (Fig. 1D) accumulated under the ice from late February to April and decreased from May to July, likely as a function of higher phytoplankton uptake following increased light availability in summer; first indications of surface NO_3^- depletion occurred in June.

Bacterial heterotrophic activity (Fig. 1E) was low but detected throughout winter and rapidly increased in late spring, parallel to the order of magnitude increase in Chl *a*. Surface bacterial abundance (Fig. 1E) followed a similar pattern with more than a 5-fold increase in cells from April to June. Additional details of bacterial activity and carbon demand during the CFL study are reported elsewhere (Nguyen *et al.,* 2012). Community composition of the bacterioplankton also changed over time (Fig. 2). Large community changes detected using DGGE, targeting the 16S rRNA gene, occurred first in April when sea-ice melt began, then in May with a summer community in May and June. In contrast, based on the DGGE results, the winter community from November to March changed very little.

4.3.2 Presence and expression of the PR gene

To characterize seasonal patterns and potential diversity of PR in Arctic samples, we used seven combinations of degenerate primers (Table S1). Two of the primer pairs that associated *in silico* to *Methylophilales* sp HTCC2181 and *Marinobacter* PR sequences did not yield PCR products (no visible bands) from any of the samples (Fig. 3A, primers E and G). The other five combinations yielded positive PCR products and positive amplification of the PR gene (from

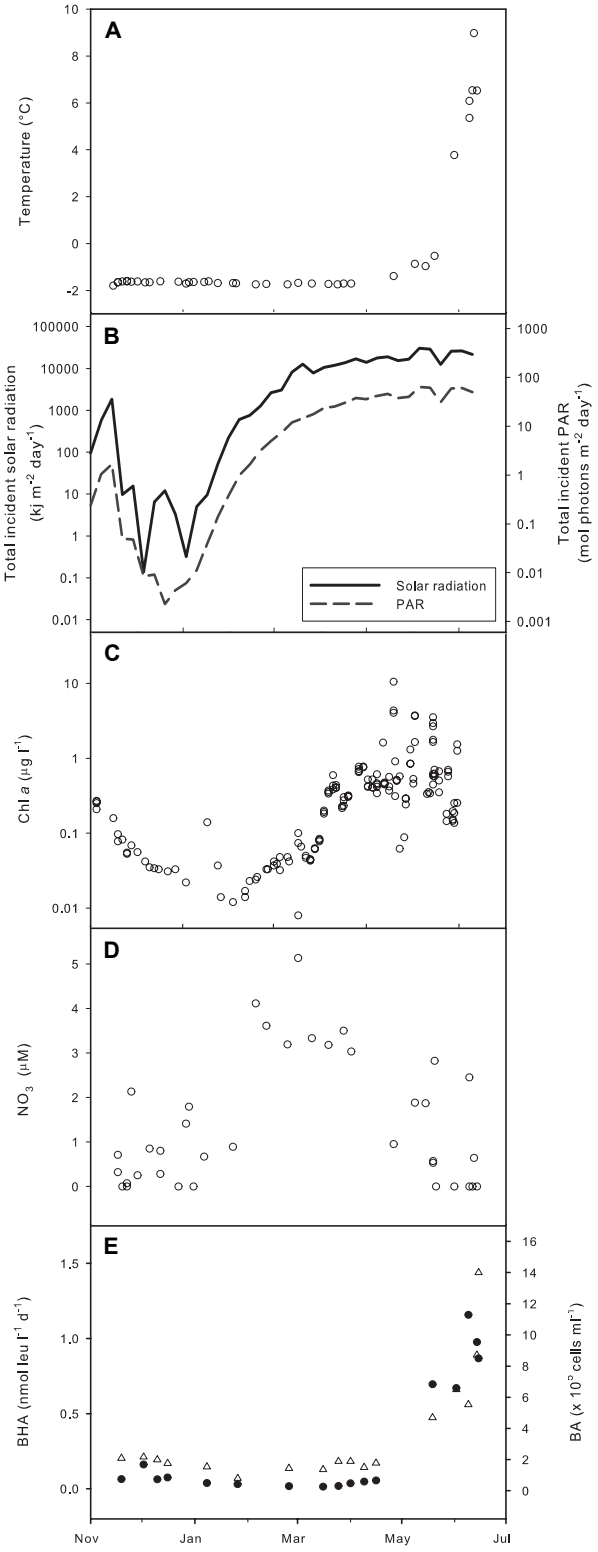

Figure 1: Temporal trends in surface water temperature (A), surface irradiance (B), Chl *a* (C), nitrate concentrations (D), and bacterial abundance (open triangles) and production (full circles, E) measured over the course of the IPY-CFL system study. Lines on the x-axis represent the first day of each month.

DNA) was observed on all dates. Primer sets A and F resulted in the greatest amplification signals which remained relatively elevated throughout the sampling period, with only a slight decrease over the winter months (Fig. 3A). Sets B and C amplification yields declined over winter, but only C appeared to re-gain relative intensity in late spring. Set D decreased during the winter months and slightly increased towards the end of the study.

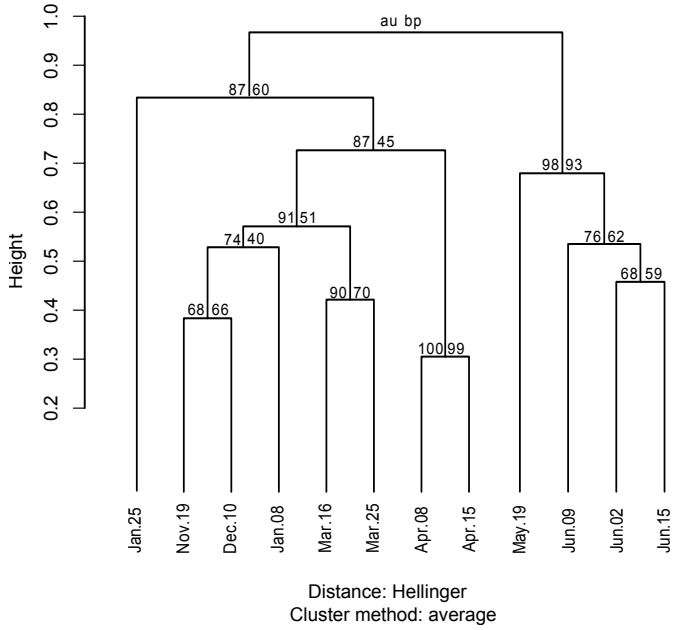

Figure 2: Patterns of diversity observed during this study illustrated as an UPGMA-based dendrogram showing seasonal variations in bacterial communities, based on cluster analysis of the DGGE fingerprints. Numbers to the left of the dendrogram branches are the approximately unbiased *p-value* (AU *p-value* in %), while numbers to the right are the normal bootstrap support (%).

For primer pairs with positive DNA amplification of PR genes (A, B, C, D, F; Table S1), we investigated the apparent gene expression using cDNA to gage PR transcript abundance in the Arctic Ocean. The intensity of the end-point PCR band was used as an estimate of that expression. All primer sets followed a similar pattern with at least one or more primer pairs with positive amplification of PR transcripts for all sampling dates (Fig. 3B). Lower apparent expression was observed during winter, with a marked decrease from December to January. Expression then peaked in April at the time of increased irradiance and ice-melt (Fig. 1B). This increase coincided with a change in the bacterial community (Fig. 2). Later in May, when the community changed again, expression decreased based on the lower intensity of PCR bands. Apparent expression progressively increased towards the end of our study, with the onset of Arctic summer (Fig. 3B). Trends linking expression patterns to environmental variables (Fig. 1) were not statistically significant although an approximate 1-

month lag between changes in environmental conditions and detected changes in PR expression was noted.

4.3.3 PR winter diversity

To gain a better understanding of seasonal distribution and diversity of PR-carrying clades, clone libraries for early (Dec.16th) and late (March 25th) winter were constructed using the five primer sets that successfully amplified PR from Arctic DNA. A total of 187 clones were

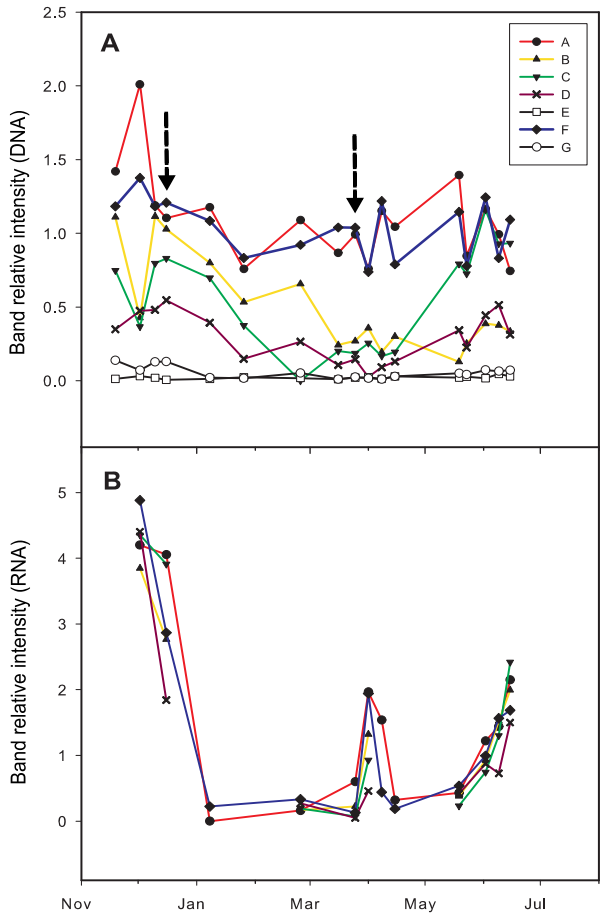

Figure 3: Change in the relative intensity of PCR bands for the multiple PR primer sets over time. A) Relative intensity observed in DNA samples and B) Relative intensity observed in RNA samples on each sampled date. The black arrows show the two dates selected for cloning and sequencing. Missing data points indicate dates where samples were either lost or in insufficient amount to allow testing of all primer sets. Lines on the x-axis represent the first day of each month. An environmental sample from the Blanes Bay Microbial Observatory time-series, where PR presence was confirmed by DNA sequencing was used as a positive control. Nanopure water was used as a negative control. Conditions were kept constant between all reactions throughout the amplification procedure. We assume that within each primer pair, all other things being equal, the differences in signal intensity can be reasonably attributed to differences in initial target DNA and cDNA.

sequenced, of which 118 were unique, and aligned against 16 PR reference sequences from GenBank (Figure S2). Phylogenetic analysis using maximum likelihood (ML) of the alignment revealed four main PR clades (Fig. S2 and Table 1): Most PR sequences were associated with Gammaproteobacteria (73% of all clones), with over half (56%) of all clones falling into the sub-clade matching Gammaproteobacteria from the Oligotrophic Marine

Group (OMG). Alphaproteobacteria and Bacteroidetes formed the other prominent clades. Finally, a small proportion of sequences remained unassigned (8% of all clones). All clades were represented at both dates, with Flavobacteria-like sequences being more abundant in early winter and more Alphaproteobacteria-like sequences in late winter (Table 1). With the exception of non-OMG Gammaproteobacteria-like sequences, all clades had strong bootstrap support (>75%, Fig. S2).

The primer pairs did not precisely match these four major clades, but some distinctive patterns of association emerged (Table 1). Primer set A produced the largest number of clones (Fig. 3A), and was associated with most of Alphaproteobacteria-like and Gammaproteobacteria-like clades. Primer set B showed similar associations with the latter clades, but produced fewer clones. Sets C and F were meant to target Flavobacteria, but they also amplified Gammaproteobacteria. F clones were the most abundant in early winter. Finally, set D was associated *in silico* to freshwater PR clades (Atamna-Ismael *et al*. 2008), and only produced 3 clones.

Operational taxonomic unit (OTU) clustering and BLAST analysis were carried out to better understand temporal patterns in Arctic PR diversity, and to test results from maximum-likelihood (ML) placements. Using MOTHUR clustering at 94% nucleotide sequence similarity (Riedel et al. 2010), 31 OTUs were identified (Fig. S3). Of these, 11 were unique to the early winter and 9 OTUs unique to the late winter, indicating a change in PR communities over that time. OTUs were assigned to bacterial classes by BLAST search using an e-value ≤1e-25 and a ≥70% maximum identity threshold. The resulting OTU clustering indicated dominance of Gammaproteobacteria-like PRs on both dates, with the second most common group, Flavobacteria-like PRs in early winter, replaced by Alphaproteobacteria-like PRs in late winter (Fig. S3).

4.4 Discussion

The Arctic Ocean, with its strong seasonal changes in irradiance, ice cover, and primary productivity, provided the perfect setting to follow *in situ* PR gene dynamics over time, and assess the potential implication of environmental factors in PR presence, expression and diversity. Overwinter access to the Arctic Ocean enabled the collection of this unique dataset covering eight consecutives months of sampling. PR was widely distributed and

Table 1. Distribution and affiliation of clone sequences in early (EW) and late winter (LW) periods, based on maximum likelihood inference and subdivided in respect to the respective primer sets. Flavo : flavobacteria, α-proteo : alphaproteobacteria, γ-proteo : gammaproteobacteria, OMG : oligotrophic marine group gammaproteobacteria.

Primer set	Flavo	α-proteo	γ-proteo	OMG	Unassigned	Total
	EW/LW	EW/LW	EW/LW	EW/LW	EW/LW	EW/LW
C1	-/-	3/12	-/11	16/46	4/10	23/79
C2	-/-	-/1	3/-	6/7	1/-	10/8
C3	3/2	-/-	2/2	11/3	-/-	16/7
C4	-/-	-/-	-/1	2/-	-/-	2/1
C6	13/2	-/-	11/1	9/5	-/-	33/8
Total	16/4	3/13	16/15	44/61	5/10	84/103

expressed throughout the entire study, although the relative intensity of expression decreased over the winter. Variations in expression levels coincided with the temporal succession of bacterial communities and lagged changes in environmental conditions. While Gammaproteobacteria-like sequences accounted for most of the diversity, the change in dominance of the secondary clade in early to late winter from Flavobacteria-like to Alphaproteobacteria-like sequences may have been related to changes in local carbon dynamics. Overall, patterns in the clones' relative abundance (Table 1) followed relative intensity patterns of apparent expression from PCRs (Fig. 3B).

4.4.1 Seasonal trends in PR presence and expression

Possession of PR would not represent an obvious a priori competitive advantage during Polar darkness given the absence of light (Béjà et al., 2001), and the photon requirements of the PR pathway. However, positive amplification for all of the PR primer combinations in all of our Arctic DNA samples from November through June suggests otherwise. While the PR gene is often reported from the photic biosphere (Finkel et al., 2013), aphotic regions appear to be under-sampled. The positive PR amplification for all primer pairs throughout the sampling period suggests that the gene's presence was not influenced by seasonally driven

environmental conditions including light availability. A previous study that detected PR genes in Polar winter samples off the coast of Barrow, Alaska, assumed PR provided some advantage to carriers (Cottrell and Kirchman 2009b), however no specific associated function was identified.

Although PR genes were detected during the darker part of the year, their expression decreased in December and winter expression was much lower than in spring and summer. The autumn-winter decrease was gradual with an approximate one-month lag between the falling irradiance and the apparent expression suggesting that PR remained useful to bacteria over that period. Although low levels of gene transcripts supports light-regulation, any expression detected during winter darkness suggests that this regulation was not complete. In addition maintaining expression over winter suggests that for some bacteria either the metabolic costs of PR synthesis are minimal, or PR is involved in processes other than light-dependent energy acquisition. The seasonal variability of PR expression suggests the gene's activity is both a function of environmental factors and changes in community structure.

Winter PR expression could be due to the availability of some light energy. Potential sources of light during the Polar darkness are bioluminescence and the Moon. Most deep-sea animals and many bacteria are bioluminescent and high levels of bioluminescence have been detected in the deep Mediterranean for periods lasting several months (Tamburini et al., 2013). This process also occurs in the Arctic, but the reported photic levels (Berge et al., 2012) are lower than those observed in the Mediterranean. When a full Moon occurs at high latitudes, it provides light over prolonged periods, but it is difficult to estimate the actual quantity of moonlight, if any, that could penetrate the snow and ice cover of the Arctic Ocean during winter. The photons generated by bioluminescence or moonlight are likely insufficient for significant energy conservation via PR proton pumping. Perhaps a differential dependence on bioluminescence or moonlight, which emit in the green to blue and visible spectrum respectively, could be inferred from the spectral tuning properties of our samples however our fragments were too short to assess this possibility. Given that PR is a relatively simple protein compared to bacterial photosynthetic pigments (e.g. BChl a) (Kirchman and Hanson 2013), thus the cost of expressing PR over winter may be minimal even when little or no ATP gains are possible. However, even when photons are limited, PR could provide indirect gains if used as a spatial or temporal biosensor (Fuhrman et al., 2008).

Even minimal light could provide an advantage to some PR bacteria, as a few key sites of rhodopsin genes have been shown to be diagnostic of sensory functions (Spudich and Jung 2005). The more recently described site A178 (Yamada et al., 2010) is of particular interest. In addition to a shift towards the red spectrum, a mutation at this position can induce a conformational change in the E and F helixes, resulting in the photocycle lasting 10 times longer (Bamann et al., 2014). In proteins with this conformational change, the binding pocket would thus remain open much longer, supporting sensory rather than ATP-generating functions, particularly in low light environments. We found that most of the sequences (79%) contained this «long photocycle» variant at site A178, and only 36 sequences had the more usual type (Ala, Cys or Thr).

The decrease in PR expression over winter, suggests some regulation in gene expression rather than being exclusively constitutive. Examples of both constitutive and regulated expression of PR have been reported; Gómez-Consarnau et al. (2007) showed increased growth and expression in response to light while Riedel and Tomasch (2010) did not, even though both studies used a marine flavobacterium (Dokdonia MED134 and PRO95, respectively). However this may have been influenced by substrate availability: the former study used low organic carbon concentrations in their experiments, whereas the latter used concentrations that were two-orders of magnitude higher. Recent studies show that nutrient availability is also important in regulating PR expression (Akram et al., 2013). Under oligotrophic conditions, increased PR expression and growth was observed in some PR carriers undergoing starvation (Gómez-Consarnau et al., 2010) and also in unamended seawater samples (Lami et al., 2009). However, a recent study (Riedel et al., 2013) did not detect a measurable growth increase in the flavobacterium PRO95, even under nutrient poor conditions mimicking oligotrophic environments. This suggests resource limitation coupled with an additional requirement of stress or starvation may be a prerequisite for measurable PR enhanced growth (Riedel et al., 2013). Although evidence for decreased respiration from light-mediated ATP-production in starved SAR11 isolates (Steindler et al., 2011) and significant light-driven proton pumping in marine Flavobacteria (Yoshizawa et al., 2012) supports the notion that PR supplements bacterial energy requirements, PR's precise physiological role and impact on carbon cycling is likely very different in different bacteria. Altogether, the presence

of PR during the Polar darkness seems to involve several mechanisms and more functional complexity than reported from in vitro experiments.

4.4.2 Winter patterns in PR diversity

During this study, we retrieved and sequenced 188 PR clones. This is an important contribution to the understanding of PR diversity in polar waters as, to our knowledge, previous polar studies yielded fewer clones (<50) (Cottrell and Kirchman 2009b, Koh *et al.,* 2010). This difference is likely a consequence of our use of more primers, 7 pairs of degenerate primers versus only 3 used in previous Arctic studies. We identified 4 main PR-carrying clades (Fig. S2) and highlighted changes in diversity from early and late winter, with almost half of the OTUs being specific to each sampling date (Fig. S3). Although degenerate primers can capture a wide range of PRs, other types of microbial rhodopsins may not be amplified (see materials and methods section). For example, our primers did not target a diverse group of rhodopsins, the actinorhodopsins, associated with Actinobacteria (Sharma et al. 2008, 2009). While diverse actinobacteria assemblages have recently been found in Arctic sediment samples (Zhang et al., 2014) and may have been underestimated in some marine regions (Gai et al. 2013), actinorhodospins are typically associated with freshwater (Sharma et al. 2008) or brackish aquatic systems (Salka et al. 2014), that are not representatives of the prevalent conditions in the sites where we sampled. Xanthorhodopsins, however, have been found to be significant contributors to microbial rhodopsin diversity predominantly in sea-ice (Vollmers et al., 2013). Unfortunately, these would not be detected with our PR primers. Future work is needed to characterize the potential contribution of both these rhodopsins in the pelagic Arctic Ocean.

A study using pyrosequencing of 16S ribosomal gene in the western Arctic reported that Gammaproteobacteria, Alphaproteobacteria and Flavobacteria were the three most abundant groups (Kirchman et al., 2010), consistent with our results. However, one might have expected Alphaproteobacteria to be better represented in our libraries, since representatives of the SAR11 are common in Arctic waters (Ghiglione et al., 2012, Kirchman et al., 2010), and are known carriers of PR (Grote et al., 2012). Given the distinctive patterns in primary production and C availability at high latitudes, changes in substrate quality likely contribute to the temporal structuring of the PR-carrying prokaryotic community.

In terms of the secondary clades, many cultured marine Flavobacteria have pathways enabling the use of high molecular weight (HMW) compounds (Fernandez-Gomez *et al.,* 2013). A relatively large proportion of the genome of a well-characterized flavobacterium (MED134), is composed of polysaccharide and protein degrading enzymes such as peptidases and glycosyl hydrolases (González *et al.,* 2011). These would facilitate use of HMW compounds when more labile, low molecular weight (LMW) organic matter is scarce, for example during post-bloom and winter periods. González et al. (2011) also identified proteins with cell-cell and cell-surface adhesion domains that would favor a particle-attached lifestyle. Particle-associated heterotrophy has been shown to be an important pathway in the coastal Amundsen Gulf, which could favor Flavobacteria (Garneau *et al.,* 2009). Additionally, BIOLOG assays have showed an important consumption of HMW compounds in December (Fernández-Gómez *et al.,* 2014, Sala *et al.,* 2008). Both observations are coherent with an increased contribution of Flavobacteria to the microbial community assemblage in early winter.

Alphaproteobacteria are competitive consumers of LMW compounds, especially amino-acids and glucose, suggesting a preferential use of LMW monomers rather than HMW polymers (Malmstrom *et al.,* 2005). Therefore, phytoplankton-derived organic matter and labile LMW monomer production in surface waters would favor Alphaproteobacteria. Given this, we were surprised to see an increased fraction of this clade in the late winter. Interestingly, pelagic Chl *a* concentration began to increase as early as February under the ice (Fig. 3), potentially explaining why Alphaproteobacteria formed a higher proportion of bacterial diversity in late winter. Furthermore, the 2008 season was characterized by an early ice-algal bloom that resulted in high sea-ice Chl *a* (> 50 µg L^{-1}) concentrations in March (Nguyen and Maranger 2011), another potential source of LMW organic matter to bacteria in surface waters. Thus, it appears possible that local patterns in organic matter lability and availability could contribute to the temporal structuring of the PR-carrying prokaryotic community.

4.5 Conclusions

Our work shows PR was widely distributed in Arctic bacterial communities and expressed even during the polar winter. Although involvement in light-activated proton pumping may be the gene's most well characterized function, low but sustained expression of the PR gene over winter suggests other functional roles. This study indicates that polar bacteria can modulate PR expression on a timescale of weeks to months in response to environmental changes. While significant benefits from PR-synthesized ATP are unlikely overwinter, its role in environmental biosensing may contribute to bacterial survival. Indeed, PR could facilitate a bacterium's ability to seek out and access limited resources. The gene remained present throughout the study despite seasonal changes in the bacterial community structure, although changes in relative expression patterns co-occurred with April and May community shifts. Changes in PR's function between the winter and spring could potentially explain this apparent synchrony. When winter PR diversity was evaluated over time, Gammaproteobacteria-like PR sequences dominated, but a transition from Flavobacteria-like to Alphaproteobacteria-like PRs occurred from early to late winter, concomitant with changes in organic matter lability and availability observed during the CFL study. These observations suggest a level of interplay between local carbon dynamics and PR-diversity patterns in Polar Oceans. Further assessment of PR's non-ATP forming pathways which need very low levels of photic energy, is required if we are to understand the impact of this gene on marine microbial dynamics and carbon economy. These will need to be paired with metabolic rates of carbon consumption by PR carriers if we are to quantify the potential impacts of PR on C cycling.

4.6 Acknowledgments

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Conflict of interest statement

The authors of this manuscript state no conflict of interest in the present work.

Supplementary information is available at ISMEJ's website

4.7 Supplementary Information

Table S1 – Primer pairs used in this study, based on Atamna-Ismael *et al.* 2008, Koh *et al.* 2010, and *in silico* testing, with associated reference strains according to *in silico* analysis.

Pair ID	**Primer ID and sequences** (5'-3' sense, 5' tail is shown in italic)	**Protein Region**	**Positive strains *in silico***
A	F1-*GCGCGGAATTC* MGN TAY ATH GAY TGG	RYIDW	*Polaribacter irgensi* 23P, *Pelagibacter ubique* HTCC1002 and HTCC1062, *Vibrio harveyi* ATCCBAA-1116, HTCC2143 (Gammaproteobacterium)
	R1-*GCGCGCAAGCTT* GGR TAD ATN GCC CAN CC	GWAIYP	
B	F1-*GCGCGGAATTC* MGN TAY ATH GAY TGG	RYIDW	HTCC2207 (SAR92 clade)
	R3-*GCGCGCAAGCTT* GGR TAD ATN SWC CAN CC	GWSIYP	
C	F2-*GCGCGGAATTC* MGN TAY GTN GAY TGG	RYVDW	Vibrio S14
	R3-*GCGCGCAAGCTT* GGR TAD ATN SWC CAN CC	GWSIYP	
D	F2-*GCGCGGAATTC* MGN TAY GTN GAY TGG	RYVDW	No positives, but suggested in Atamna-Ismael *et al*. (2008)
	R2-*GCGCGCAAGCTT* GGR TAD ATN ACC CAN CC	GWVIYP	
E[1,2]	F2-*GCGCGGAATTC* MGN TAY GTN GAY TGG	RYVDW	Methylophilales HTCC2181, *Marinobacter* ELB17
	R4-*GCGCGCAAGCTT* GGR TAD ARN SHC CAN GA	SW**T**FY/SW**A**FY	
F	F2-*GCGCGGAATTC* MGN TAY GTN GAY TGG	RYVDW	*Dokdonia* MED134, *Polaribacter* MED152, Flavobacterium BAL38, Photobacterium SKA34
	R1-*GCGCGCAAGCTT* GGR TAD ATN GCC CAN CC	GWAIYP	
G[1]	F3-*GCGCGGAATTC* MGN TAY GCN GAY TGG	RYADW	No positives, but suggested in Koh *et al*. 2010 and Atamna-Ismael *et al*. 2008
	R3-*GCGCGCAAGCTT* GGR TAD ATN SWC CAN CC	GWSIYP	

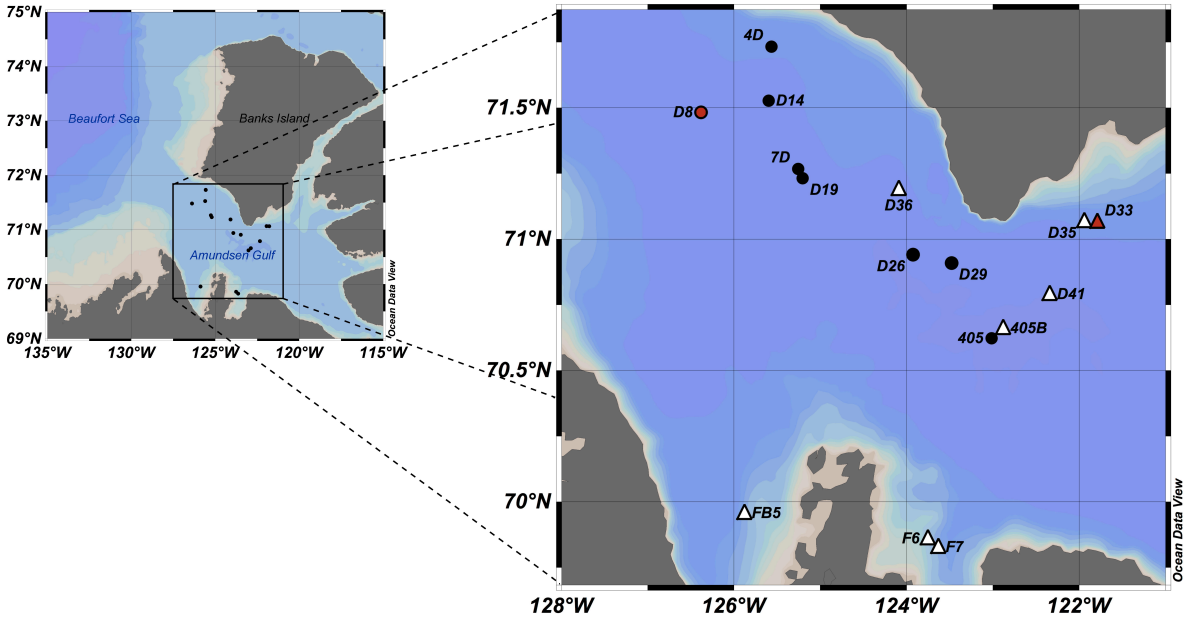

Figure S1 : Map of the Amundsen Gulf region and sampled stations. Dots represent the Winter sites, while triangles represent the Spring sites. Red symbols are sites that were chosen for cloning and sequencing.

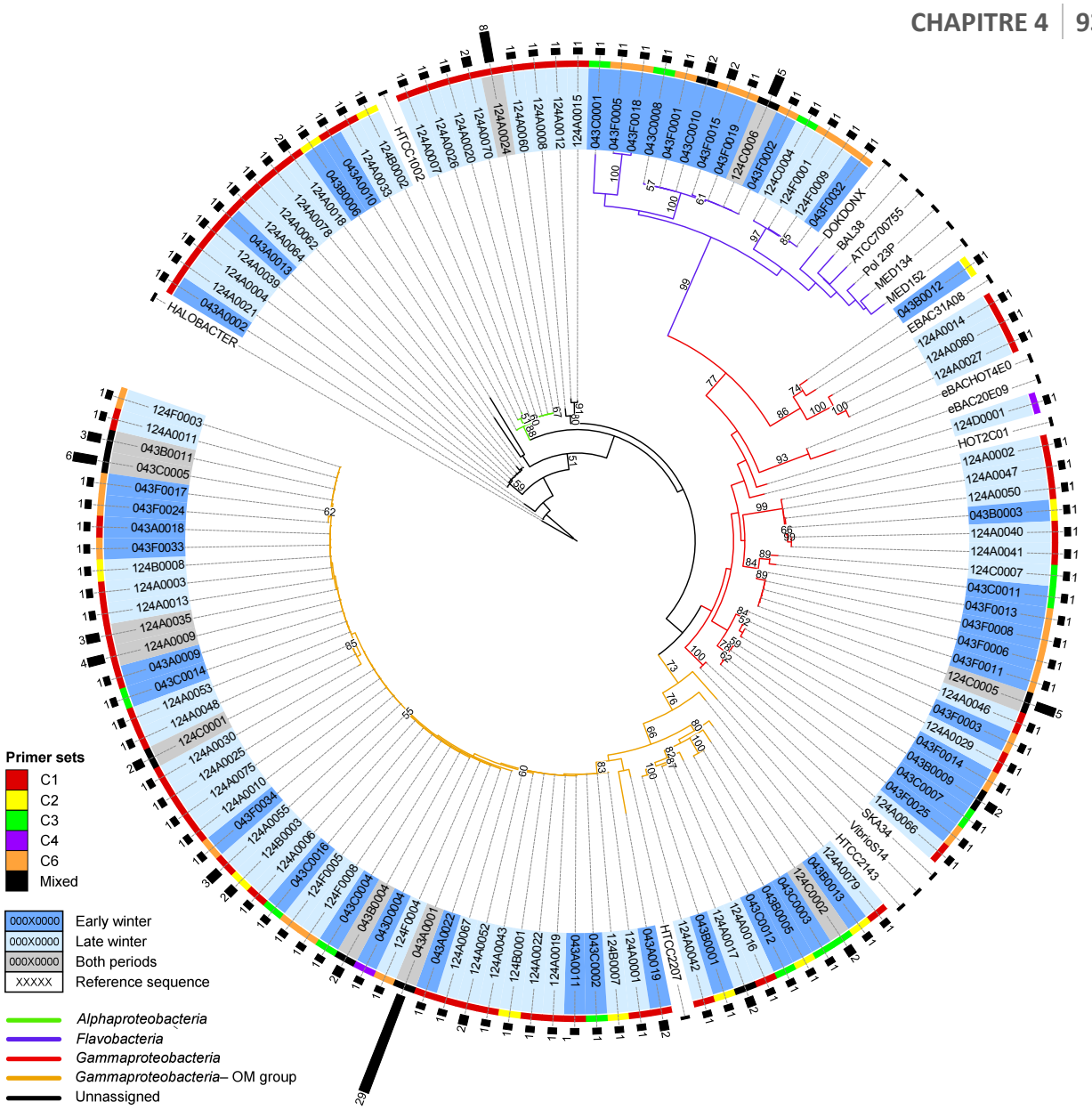

Figure S2: Phylogenetic tree based on DNA sequences of early winter and late winter sites, generated with maximum likelihood inference. Bootstrap values higher than 50% are shown on branches. The color of branches indicates affiliation to bacterial genera, the color strip refers to the associated lettered primer sets, and the leaf color to the period of collection (both, early or late winter). The number of affiliated sequences is presented as bar charts. Reference sequences of marine proteorhodopsin genes (white leaves) were found in GenBank. Corresponding NCBI GIs and taxonomical details are as follows in alphabetical order: Alphaproteobacterium HOT2C01 [GI:37913013 – Alphaproteobacteria.]; *Cand. Pelagibacter ubique* HTCC1002 [GI:91762240 – Alphaproteobacteria; SAR11 cluster; Candidatus Pelagibacter.]; *Dokdonia donghaensis* MED134 [GI:86130673 – Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Dokdonia.]; *Dokdonia sp.* PRO95 [GI:223452819 – Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Dokdonia.]; Flavobacteria bacterium BAL38 [GI:126663919 – Flavobacteriia, Flavobacteriales.]; Gammaproteobacterium EBAC20E09 [GI:45644626 – Gammaproteobacteria; SAR86 cluster.]; Gammaproteobacterium EBAC31A08 [GI:9971913 – Gammaproteobacteria.]; Gammaproteobacterium eBACHOT4E07 [GI:47779380 – Gammaproteobacteria; SAR86 cluster.]; Gammaproteobacterium HTCC2143 [GI:119476620 – Gammaproteobacteria; OMG group; BD1-7 clade.]; Gammaproteobacterium HTCC2207 [GI:141534101 – Gammaproteobacteria; OMG group; SAR92 clade.]; *Photobacterium angustum* S14 [GI:90580458 – Gammaproteobacteria; Vibrionales; Vibrionaceae; Photobacterium.]; *Photobacterium sp.* SKA34 [GI:89074634 – Gammaproteobacteria; Vibrionales; Vibrionaceae; Photobacterium.]; *Polaribacter irgensii* 23-P [GI:88802358 – Flavobacteriia, Flavobacteriales, Flavobacteriaceae; Polaribacter.]; *Polaribacter sp.* MED152 [GI:85819768 – Flavobacteriia, Flavobacteriales, Flavobacteriaceae; Polaribacter.]; *Psychroflexus Torquis* ATCC 700755 [GI:91216393 – Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Psychroflexus.]. *Halobacterium salinarum* (strain Shark) [GI:461612 – Halobacteria; Halobacteriales; Halobacteriaceae; Halobacterium.] was used as the outgroup. All clone sequences used to build this tree are deposited in GenBank under accession numbers KJ937475-KJ937662.

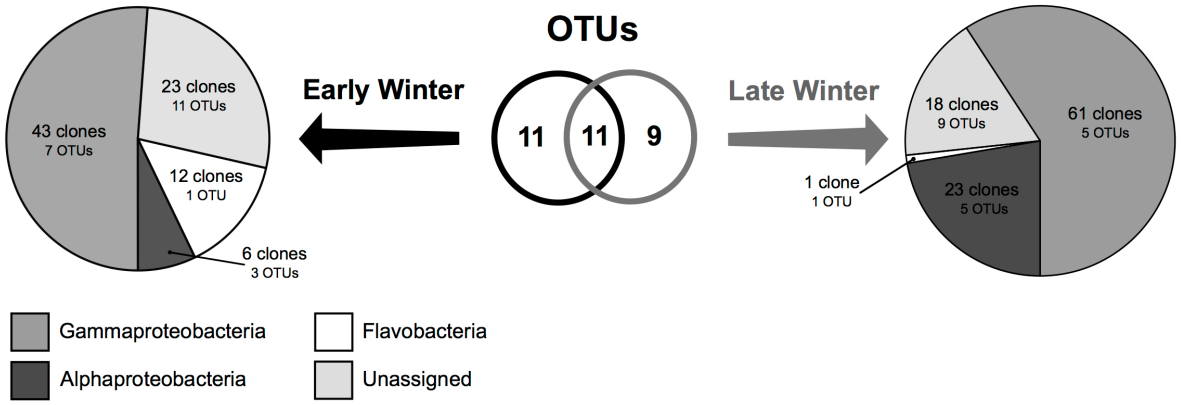

Figure S3: Number and distribution of PR OTUs between the samples sequenced in early and late winter. The Venn diagram shows the distinct and shared distribution of OTUs between dates, while the pie charts show the number of OTUs and clones associated to each bacterial class.

Chapitre 5 : Conclusions

5.1. Le métabolisme microbien arctique

Tout au long de cette thèse, nous avons cherché à déterminer si l'activité métabolique microbienne des glaces et des eaux arctiques, particulièrement la respiration, pouvait consommer une part importante des ressources disponibles en Arctique, et ce tout au long de l'année. L'intérêt de cette question résidait dans le fait que la découverte d'une boucle microbienne plus active qu'anticipée pourrait affecter les transferts énergétiques vers les maillons trophiques supérieurs et, ultimement, la balance métabolique du système.

Nous envisagions deux scénarios principaux. D'un côté, l'activité métabolique est surtout déterminée par la production bactérienne qui permet un stockage d'énergie dans la biomasse bactérienne. Celle-ci permet un recyclage d'énergie vers le reste du réseau trophique par les bactérivores. Sans la production bactérienne, la majorité de cette énergie serait perdue. De l'autre côté, l'activité métabolique est dominée par la respiration, qui limite les transferts potentiels vers les maillons trophiques supérieurs. Comme la respiration consomme le C sans

Photo: Retraite des glaces lors de la fonte printanière, Golfe d'Amundsen, Juin 2008

produire de biomasse, cette énergie n'est pas transférable au reste du réseau trophique. C'est pourquoi une respiration importante amène généralement une diminution de l'efficacité énergétique globale d'un système. C'est avec ces hypothèses en tête que nous nous sommes joints au projet CFL-IPY. Par des mesures répétées de l'activité bactérienne et de la respiration sur plus de 9 mois, nous avons amassé un jeu de donnée unique, un des rares à dresser un portrait saisonnier de la consommation de C par les communautés microbiennes arctiques. Nos travaux, tant sur la banquise qu'en zone pélagique, démontrent que la respiration est le processus dominant, malgré l'atteinte de taux appréciable de production bactérienne au printemps. Nos observations suggèrent un recyclage interne important de la PP locale pour alimenter l'ensemble du réseau trophique.

Dans un second temps, nous avons tenté de mieux caractériser l'écologie des bactéries arctiques en ciblant un gène spécifique, celui de la protéorhodopsine (PR). Cette protéine représente une alternative aux voies métaboliques hétérotrophiques classiques et permet un apport d'énergie supplémentaire par la capture de l'énergie solaire. Son implication dans des fonctions sensorielles ou de détection pourrait fournir un avantage compétitif au bactérium, même lorsque les niveaux photiques sont trop faibles pour entraîner un gain énergétique net. À notre connaissance, aucune étude n'a effectué un suivi temporel de la présence et de l'activité du gène chez les communautés naturelles, spécialement dans les milieux polaires dont l'accessibilité demeure limitée. La motivation derrière ces efforts était de tenter d'identifier de nouvelles pistes de réflexion pour les recherches futures sur le métabolisme microbien en Arctique. En effet, la diversité fonctionnelle des bactéries est grande alors que notre connaissance de sa distribution et de son potentiel écologique est limitée à seulement quelques voies métaboliques classiques. Par une approche métagénomique, nous explorons la distribution et l'expression de la PR dans les communautés microbiennes arctiques sur une période de 8 mois. Nos résultats montrent la présence et l'expression soutenues du gène au travers des saisons chez les communautés bactériennes arctiques. Nous montrons que le gène de la PR est présent et actif chez des communautés diversifiées. Il reste à voir si la fonction du gène – soit énergétique ou sensorielle – peut être quantifiée et intégrée aux modèles trophiques dans le futur.

5.2. Des microbes sur glace, un cocktail de répercussions

Les glaces de l'Arctique sont devenues une figure emblématique des changements climatiques. Année après année, de nouveaux records d'étendue minimale de la banquise sont battus, le dernier datant de 2012 (NSIDC). Étonnamment, malgré une abondance de données sur la dynamique physique et chimique de la banquise, les données microbiologiques disponibles pour la glace de mer sont rares. Lorsque présentes, elles se limitent souvent à une relation positive entre l'abondance bactérienne et les concentrations en composés dissous (Bunch et Harland 1990, Kahler *et al.,* 1997, Riedel *et al.,* 2007). Les études plus récentes tendent à mettre la diversité des communautés de glaces et leur production de substances exopolymériques au centre de leurs préoccupations (Collins *et al.,* 2010, Eronen-Rasimus *et al.,* 2014, Underwood *et al.,* 2013) alors que très peu d'études ont cherché à mesurer les taux d'activité microbienne des glaces de mer.

En 2008, des participants au projet CFL ont remarqué des concentrations et des flux de CO_2 dans la glace trop grands pour n'être expliqués que par les facteurs physiques (Papakyriakou *et al.,* 2011, Miller *et al.,* 2011). Nous croyons que ce flux peut être expliqué par la forte activité bactérienne dans la glace, consommant beaucoup de C et le relâchant sous forme de CO_2 par la RB. En effet, l'activité des bactéries a été démontrée dans la glace (Junge *et al.*, 2004) et les taux élevés de BP mesurés font croire que les glaces de mer s'apparentent plus aux biofilms microbiens qu'aux communautés microbiennes marine typiques (Kaartokallio *et al.,* 2013). Toutefois, aucune étude n'a mesuré directement la respiration dans ces glaces. Nos travaux montrent que les communautés bactériennes sont à même de profiter de ces apports et de croître activement dans les glaces de mer. Les fortes concentrations de matières organiques semblent même stimuler la biosynthèse aux dépens de la respiration, amenant une hausse appréciable de l'efficacité de croissance dans les glaces de mer.

Les glaces de mer peuvent constituer un site important de photosynthèse par les algues de glaces qui, bien que sur une courte période, peuvent représenter une proportion importante du carbone organique disponible pour le réseau trophique (Gosselin *et al.,* 1999). Néanmoins, le ratio RC :PP de 16 observé entre les mois de février et mars, où la PP était faible et la RC relativement stable, suggère une consommation soutenue de C dans la glace. Ces résultats sont

en accord avec des estimations récentes montrant la respiration comme le processus dominant en hiver (Fransson *et al.,* 2013). De plus, cette étude montre que l'accumulation hivernale de CO_2 dans les glaces est une source nette de C inorganique dissous (CID) pour les eaux sous-jacentes durant la fonte printanière. De plus, il semble que la respiration pourrait être plus sensible que la PP aux hausses des températures (Kritzberg *et al.*, 2010, Vaquer-Sunyer *et al.*, 2012), telles que prévues en Arctique. Dans ce contexte, il reste à déterminer comment l'interaction entre fonte saisonnière hâtive de la banquise et hausse potentielle des taux de respiration influencera le bilan métabolique des glaces de mer.

5.3. L'Arctique : source ou puits de carbone?

Le rôle important des microorganismes pélagiques et leur contribution au cycle du carbone sont connus en Arctique (Meon et Amon 2004, Garneau *et al.*, 2008, Cottrell *et al.*, 2006, Kirchman *et al.*, 2009) mais surtout limité aux données de production bactérienne et durant la saison estivale. Ainsi, nous en savons très peu sur l'activité microbienne pour la majeure partie de l'année. De plus, alors que la majorité du C consommé par les bactéries est catabolisée durant la respiration, les mesures directes de la respiration et d'efficacité de croissance sont rares, même inexistantes dans le cas du golfe d'Amundsen. En effet, les études précédentes réalisées dans la région n'ont pas mesuré directement la respiration et l'ont plutôt estimée à partir de la PB en utilisant une ECB fixe (15-27 %). Ces travaux concluaient à la nette hétérotrophie de la Baie de Franklin (Garneau *et al.*, 2008). Kirchman *et al.*, (2009) ont plus récemment mesuré directement des ECB faibles, de l'ordre de 7 % dans la mer de Chukchi, suggérant une surestimation de l'ECB en Arctique. Ainsi, la quantité de C consommée par les bactéries et perdue par la respiration serait sous-estimée et nécessiterait des apports allochtones importants pour soutenir le métabolisme bactérien du golfe d'Amundsen.

Nos travaux explorent, pour la première fois, les patrons de respiration et d'efficacité de croissance bactérienne sur une période de plus de 9 mois consécutifs. Cette période inclut la formation de la banquise en automne, la nuit polaire hivernale, la fonte printanière et le passage à la période estivale des eaux libres. Notre présence en Arctique durant toute cette période nous a permis de récolter un jeu de donnée unique sur l'activité de la boucle

microbienne en Arctique et de son importance dans les transferts d'énergie à la chaîne trophique classique. Nous montrons une intensité surprenante et relativement stable de la respiration, qui perdure tout au long de l'hiver, malgré la faible disponibilité de substrats. De ce fait, ce sont les changements saisonniers dans les taux de production bactérienne qui sont déterminants dans l'efficacité de croissance bactérienne, avec des hausses importantes suivant le redémarrage de la PP au printemps. C'est également à ce moment que des transferts significatifs de C bactérien vers les maillons trophiques supérieurs semblent le plus probables. Toutefois, il semble que les températures froides et la faible disponibilité de la matière organique amènent une utilisation peu efficace de la PP par les communautés microbiennes de la région (Ortega-Retuerta *et al.*, 2012). Une observation cohérente avec nos données sur la persistance d'une forte respiration durant toute l'année, menant à un bilan métabolique hétérotrophe pour l'écosystème. Finalement, les hausses importantes de température observées en Arctique (figure 5.1), s'approchent du seuil critique, établi à une hausse de 5.4°C, où un passage rapide vers la nette hétérotrophie est anticipé (Holding *et al.*, 2013), ce qui pourrait suggéré une exacerbation du déséquilibre avec les changements climatiques.

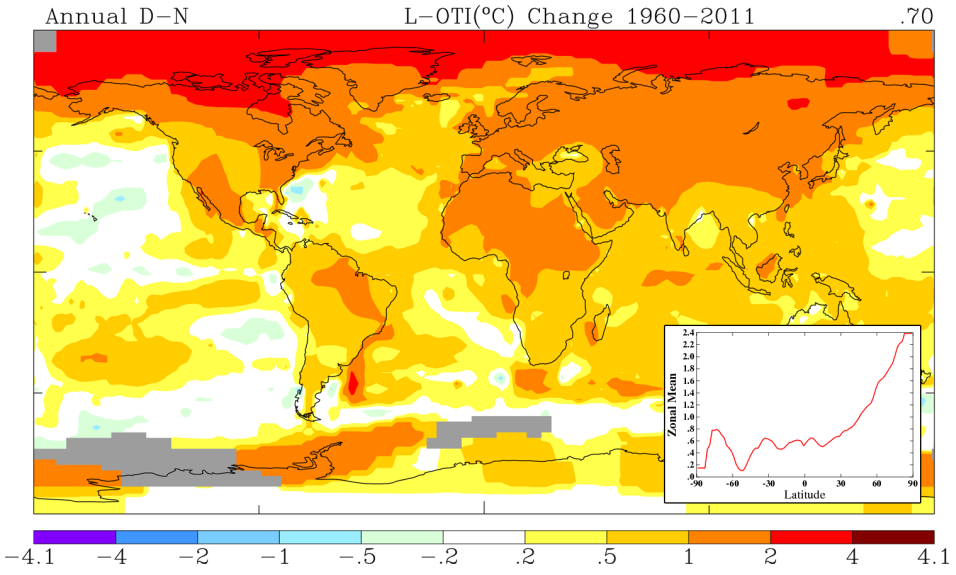

Figure 1 : Hausse globale observée des températures de surface moyennes pour la période 1960-2011. Source : NASA, Goddard Institute for Space Studies.

Malgré le rôle important des apports allochtones pour l'océan Arctique, ceux-ci semblent insuffisants pour alimenter la grande demande en C de la composante microbienne. Nous proposons l'existence d'un recyclage interne rapide de la production locale, à des échelles temporelles inférieures à nos méthodes de mesures, et expliquant l'apparent déficit en C du système. Dans un échantillonnage à haute fréquence *in situ* du transcriptome des microbes hétérotrophes, Ottesen *et al.* (2013) observent des variations du transcriptome qui supporterait cette rapidité d'adaptation des communautés hétérotrophes. Ainsi, ils montrent des patrons multi-taxonomiques de régulation génomique qui reflètent des réponses sporadiques et à court terme à la haute variabilité de l'environnement des microorganismes hétérotrophes. Ainsi, peu importe leur espèce, ces communautés ajusteraient leur machine métabolique de façon synchrone en réaction à des changements rapides des conditions environnementales locales. Ces observations suggèrent qu'il existe toute une gamme de processus à court terme que nous avons omis dans les modèles actuels. Bien que ces processus à court terme n'aboutissent pas nécessairement à des entrées ou sorties supplémentaires de C, ils pourraient expliquer les déséquilibres rencontrés occasionnellement durant l'estimation de la balance métabolique des systèmes aquatiques. Nous croyons qu'avec le raffinement des méthodes de mesures de l'activité métabolique microbienne, notamment en sensibilité et en rapidité, les recherches futures seront en mesure d'évaluer l'influence de ces processus à microéchelle sur les tendances observées à l'échelle de l'écosystème.

5.4. L'impact potentiel de la photohétérotrophie

La caractérisation de la protéorhodopsine (PR) est une des premières avancées en génomique marine ayant permis d'établir une relation entre la structure des communautés et leur fonction (Béjà *et al.* 2000, Gómez-Consarnau *et al.*, 2007, 2010). La PR, en association avec le chromophore rétinal, permet de créer un potentiel chimiosmotique entre la membrane cellulaire et l'environnement à partir de la lumière. Ce potentiel peut ensuite être redirigé vers d'autres processus cellulaires et servir, par exemple, à la mobilité flagellaire, à des processus de transport la synthèse d'ATP ou encore à des fonctions de détection (Furhman *et al.*, 2008, DeLong et Béjà 2010). Cette capacité à capturer l'énergie lumineuse pourrait ainsi conférer un avantage compétitif aux communautés présentant le gène de la PR.

Les données génomiques demeurent rares en Arctique, principalement en raison des nombreux défis logistiques associés aux recherches en régions polaires. De plus, les études génomiques sont axées sur la structure phylogénétique des communautés des régions arctiques (Garneau *et al.*, 2008, Galand *et al.*, 2008, Kirchman *et al.*, 2010), ou, dans le cas de la PR, basées sur des lignées bactériennes isolées et cultivées (Dokdonia *sp.* MED134, Polaribacter *sp*. MED152, Gómez-Consarnau *et al.*, 2007; PRO95, Riedel *et al.*, 2011, 2013). Ainsi, nous avons très peu d'information sur le rôle de gènes fonctionnels chez les communautés *in situ*. Avec le développement de la métagénomique et la hausse de nos capacités de séquençage, nous pouvons désormais sonder directement les assemblages bactériens naturels, non cultivés, pour la présence et l'expression de gènes spécifiques. C'est d'ailleurs le souhait énoncé par un groupe de chercheurs dans une publication récente portant sur la distribution des rhodopsines dans l'environnement :

> «Même si les données disponibles suggèrent que la majorité des cellules procaryotiques de la biosphère photique ont un potentiel phototrophique, et que plusieurs contiennent des gènes de la rhodopsine, une estimation précise de leur réelle expression et activité doit être réalisée au niveau de la protéine et fonctionnel. Nous espérons que les profils intrigants suggérés par nos analyses encourageront des mesures plus justes de l'activité des rhodopsines en nature.» (Finkel *et al*. 2013, traduit de l'anglais)

Ce court extrait illustre bien la pertinence de nos résultats. En effet, notre étude est l'une des rares à avoir suivi, sur une longue période (8 mois consécutifs), non seulement la présence du gène de la PR mais également l'expression de celui-ci en milieu naturel. Les amorces utilisées dans la présente étude permettent de cibler une plus grande diversité du gène de la PR grâce à quelques nucléotides «dégénérés» dans certaines régions clés de leur séquence. Bien qu'elles ne soient pas universelles, ceci leur confère une certaine tolérance à la variabilité naturelle du génome bactérien. Dans cette étude, comme la présence et l'expression du gène étaient incertaines, cinq paires d'amorces dégénérées furent utilisées pour maximiser notre potentiel d'amplification du gène. Ceci explique peut-être pourquoi nous sommes parvenus à détecter un signal clair, continu entre novembre et juin, et à isoler un nombre appréciable de clones. Le gène semble non seulement présent, mais activement exprimé par les bactéries arctiques. Finalement, nous observons une transition entre Bacteroidetes et Alphaprotéobactéries du

début à la fin de l'hiver, qui concorderait avec les variations locales des sources de C mesurées durant le projet CFL.

Il reste maintenant à quantifier l'importance de la PR dans la consommation de C des procaryotes. Jusqu'à présent seulement certaines études sont parvenues à mesurer des gains en croissance lorsqu'une culture porteuse du gène était exposée à la lumière (Gómez-Consarnau *et al.*, 2007, Akram *et al.*, 2012). Toutefois, il demeure difficile de mettre un chiffre sur l'économie de C réalisée par l'utilisation de la PR. En permettant de calculer l'abondance d'un gène, le développement des méthodes d'amplification quantitatives (qPCR) sera instrumental à la meilleure quantification des impacts de la PR sur les flux de C. De plus, au fur et à mesure des avancées dans la caractérisation du gène, le développement d'amorces ciblant spécifiquement les régions du gène associées à ses diverses fonctions contribuera à une meilleure identification du rôle de la PR en milieu naturel. En effet, il importe de rappeler que le gène peut remplir des fonctions sensorielles dont les gains seraient difficilement mesurables, malgré leur contribution probable à la survie des microorganismes. De là l'importance de continuer à mieux définir l'écologie des microorganismes et les facteurs favorisant leur prolifération. Avec les moyens dont la science dispose présentement, est-il possible de se mettre «dans la peau» d'un microbe et de conceptualiser les interactions entre la matière et les communautés qui l'entourent, à des échelles spatiales et temporelles complètement différentes des nôtres? Des travaux récents ont souligné l'importance d'ajuster nos cadres conceptuels à ce monde microscopique soumis à des variations à des échelles de l'ordre de la milliseconde et du micromètre (Stocker 2012). Une multitude de questions demeurent toujours sans réponses. Par exemple, est-ce que le gène est présent essentiellement chez les communautés actives, chez celles en dormance ou indifféremment chez l'une et l'autre? Si majoritairement présent chez les bactéries actives, on pourrait croire que la PR favorise la croissance active des bactéries, une indication d'un avantage compétitif chez les porteurs du gène. Et surtout, comme la portion active est responsable de la majorité des flux biogéochimiques, c'est dans cette situation que son impact sur le cycle du C de l'océan Arctique serait le plus grand.

5.5. Limitations des méthodes de mesure de la respiration

Les travaux de recherches, en particulier en Arctique, demandent une part de compromis, tant au niveau humain, logistique ou méthodologique. Tout au long de nos travaux, en utilisant les connaissances les plus à jour, nous avons tenté de trouver le meilleur compromis pour répondre à nos objectifs de recherche. Ces choix ont imposé certaines limitations que nous souhaitons souligner dans la présente section. Nous proposons également des solutions concrètes pour parfaire les méthodes utilisées, et nous espérons que ces recommandations pourront servir de base pour approfondir notre connaissance du métabolisme microbien polaire.

5.5.1 Mesure de l'activité bactérienne dans les glaces et les eaux du golfe d'Amundsen.

Nos travaux mesurant l'activité microbienne dans l'eau et les glaces de mer étaient soumis à quatre contraintes majeures : 1) l'espace et le temps limité à bord, 2) le besoin de mesurer les taux dans la phase liquide, 3) notre équipe restreinte, 4) l'absence de données ou de méthode de mesure des processus respiratoires dans les glaces de mer au moment de l'échantillonnage. De plus, la nature collaborative du projet CFL nécessitait une part de négociation au niveau des installations communes, notamment pour les exigences en température des chambres froides. Ces contraintes ont en grande partie guidé nos choix méthodologiques.

Il importe de rappeler que les glaces de mer sont un milieu hétérogène, dont les caractéristiques physicochimiques peuvent varier dans le temps, le site et la section de la glace considérée. En effet, d'importants gradients existent entre la base de la glace en contact avec les eaux de surface (glaces inférieures) et la glace à la surface de la banquise (glaces supérieures), en contact avec la neige et l'atmosphère (Petrich et Eicken, 2010). Les glaces supérieures sont en général plus froides et denses que les couches inférieures. Il en résulte une plus faible concentration de canaux saumâtres dans ces sections et, en général, une plus faible activité bactérienne (Deming, 2010). De plus, les canaux des parties supérieures pourront atteindre des salinités importantes à mesure que les sels sont purgés de la glace solide. À l'opposé, les glaces inférieures sont caractérisées par des températures et salinités plus proches de celles de l'eau de mer. Ces conditions seront favorables à l'établissement d'un réseau plus

abondant de canaux saumâtres à la base de la glace. Les canaux, qui abritent la majorité des organismes des glaces, seront le site d'une importante activité biologique autant hétérotrophe qu'autotrophe et ce, particulièrement au printemps. À défaut de posséder des moyens logistiques suffisants pour mesurer les taux dans la totalité de la glace, les caractéristiques susmentionnées ont motivé notre choix de cibler la base de la glace, pour maximiser notre détection des transformations biologiques du C. Considérant la nature poreuse et hétérogène des glaces de mer, une part importante des flux observés à la surface des glaces de mer dépend du bilan des réactions biologiques se déroulant dans les couches inférieures.

Nos méthodes de mesure nécéssitaient également un échantillon en phase liquide. Ainsi, nous ne pouvions pas contourner la nécessité de faire fondre les échantillons de glace, qui s'accompagne d'une perte de son hétérogénéité naturelle. C'est pourquoi, humblement, nous présentons nos taux comme des taux potentiels, que nous considérons comme un estimé des limites supérieures des taux observables dans la glace *in situ*. Nous avons utilisé des pratiques éprouvées pour minimiser les impacts de la fonte sur les communautés biologiques. Entre autres, la dilution dans l'eau de mer filtrée pour éviter le choc osmotique associé à une diminution rapide de la salinité (Garrison et Buck, 1986, Thomas *et al.*, 2010). La fonte a l'avantage de permettre une certaine homogénéisation des taux, ce qui permet de les rapporter sur une base volumique (taux par l^{-1}) ou de surface (taux par m^{-2}). Ainsi, malgré les limitations mentionnées ci-dessus, ces taux obtenus à partir d'échantillons fondus demeurent facilement extrapolables à de plus grandes étendues contrairement à d'autres méthodes. Par exemple, lors de la collecte de l'eau des canaux saumâtre, réalisée par le perçage d'un trou partiel dans la glace où les canaux se déverseront (en anglais : «*sack hole*»), il est presque impossible de déterminer l'aire drainée par les canaux et d'extrapoler les valeurs à une surface ou un volume de glace (Deming, 2010).

Certaines méthodes permettraient d'obtenir une meilleure représentativité des taux mesurés dans la glace. Ainsi, avec l'amélioration des instruments de mesure en continu, l'incubation *in situ* de carottes de glace en utilisant des optodes sensibles à l'O_2 est possible. Deux carottes de glace (ou sections de celle-ci) pourraient être insérées dans deux cylindres hermétiques munis d'un ou plusieurs capteurs qui suivraient les changements en O2. En utilisant un cyclindre imperméable aux radiations solaires et un autre transparent, la

respiration nette et brute seraient plus facilement déterminées. Les tubes seraient ensuite ré-insérés aux sites de collecte des carottes pour la durée de l'incubation. Cette méthode permettrait de résoudre plusieurs biais soit, entre-autres : 1) la besoin de recourir à la fonte, 2) les difficultés à extrapoler à une aire ou un volume plus étendus, et 3) les difficultés à reproduire en laboratoire la température et l'irradiance *in situ*. Ces mesures devraient être accompagnées de données sur les nutriments, la matière organique et l'abondance bactérienne au début et à la fin de l'expérience pour permettre une interprétation plus exhaustive des résultats. Il serait également envisageable de tester la contribution respective des portions supérieures et inférieures de la glace aux flux de C, dans la mesure où nos méthodes seront assez sensibles pour les détecter.

5.5.2 Respiration, efficacité de croissance bactérienne et l'implication des facteurs de conversion

Plusieurs choix méthodologiques ont été effectués afin d'obtenir les taux de respiration et les efficacités de croissance bactérienne (ECB) les plus valables possibles en fonction des contraintes imposées par un échantillonnage en Arctique. Dès le départ, nous n'avons pas préfiltré nos échantillons pour éviter de retirer une proportion importante des communautés microbiennes potentiellement associées aux particules (Garneau *et al.,* 2009, Kellogg *et al.,* 2011), ce qui aurait pu compromettre l'ensemble de nos mesures. Nous avons plutôt utilisé une relation empirique (Robinson 2008) pour convertir la respiration des communautés microbiennes en respiration bactérienne. Cette relation est basée sur de multiples études en milieux marins et semble être en accord avec d'autres travaux réalisés en Arctique de l'ouest (Kirchman *et al.*, 2009a), lorsque les valeurs plus élevées sont incluses. La mesure concluante de la respiration a demandé des incubations plus longues qu'anticipées, rendant la relation entre nos mesures plus courtes de production bactérienne (PB), moins évidente. Il semble toutefois que les mesures de respiration soit moins sensibles à une augmentation du temps d'incubation (Robinson 2008), suggérant un impact limité sur nos résultats. Par précaution, nous avons choisi de présenter les valeurs de respiration et d'ECB comme des taux potentiels. Néanmoins, notre étude demeure une des rares à avoir mesuré sur une période étendue la respiration. Nous sommes confiants que nos mesures sont d'une grande valeur pour notre compréhension des flux biologiques de C en Arctique.

Nos travaux nous ont permis de constater l'importance de plusieurs paramètres lors de la mesure du métabolisme microbien. Nous soumettons ici quelques recommandations pour accroître la précision des mesures de ces processus en milieu polaire. Tout d'abord, le contrôle de la température est primordial. Beaucoup d'attention doit être portée à l'adaptation des espaces de laboratoire pour un contrôle efficace et simple de la température et permettant des incubations à diverse températures simultanément. Et ce, même lorsque le navire est en mouvement et la mer agitée. Deuxièmement, nous recommandons de répéter les mesures de PB et d'abondance à la fin des mesures de respiration pour mieux suivre les effets de la durée d'incubation sur les communautés bactérienne. Un suivi génétique des changements des communautés procaryotiques durant les incubations pourrait également être envisagé pour un sous-groupe d'échantillons. La mesure en continu des concentrations en O_2 durant les incubations amènera une diminution des temps d'incubation. Cette dernière optimisation autoriserait une réallocation des ressources matérielles vers une comparaison des taux en eau filtrée vs non-filtrée, menant à une meilleure caractérisation de la contribution bactérienne à la respiration microbienne totale. Toutes ces mesures auront pour effet de diminuer l'utilisation de facteurs de conversions empiriques et d'optimiser l'utilisation des ressources et dresser un portrait plus précis de l'activité microbienne en Arctique.

5.6. Perspectives

Nous sommes parvenus à démontrer une activité microbienne soutenue en Arctique et, pour la première fois, que la respiration représentait une perte majeure de C organique pour le golfe d'Amundsen. En nous basant sur la présence, l'expression et le séquençage du gène de la PR, nous montrons également qu'une grande diversité de communautés microbienne exprime la voie photohétérotrophique, autant en hiver qu'en été. Ces résultats ouvrent la voie à des travaux futurs qui, nous espérons, prendront en considérations nos conclusions et les quelques suggestions qui suivent avant de s'engager dans leurs études.

Tout d'abord, le rôle de la température doit être mieux défini. Déjà, des travaux sont en cours dans notre laboratoire pour élucider cette question (Labrie et al. en préparation), mais beaucoup de travail reste à faire. Le seul fait de contrôler adéquatement la température sur un navire en mouvement où le temps et l'espace sont limités représente parfois un défi de taille.

Les travaux futurs devront porter une attention particulière à ce paramètre. Une meilleure compréhension de la variation des Q_{10}, et des effets du réchauffement sur les communautés in situ, particulièrement les effets différentiels sur la PP et la respiration, seront essentiels pour prédire leurs conséquences sur le bilan de C des systèmes arctiques.

La respiration microbienne n'est pas la seule composante de la boucle microbienne sous-représentée dans la littérature et les bases de données arctiques. En effet, malgré des indications du rôle important de la lyse virale et de la bactérivorie dans les transferts d'énergie microbiens dans l'Arctique de l'ouest (Maranger et al., soumis à Oceanography), seuls quelques laboratoires dans le monde s'attardent à ces processus en Arctique. Ces derniers peuvent exercer un grand contrôle sur les communautés procaryotiques. Toutefois, le manque d'études à plus grande échelle spatio-temporelle limite notre capacité à quantifier adéquatement leurs impacts pour déterminer leur importance dans les flux biogéochimiques régionaux. Les recherches futures devront incorporer ces mesures si nous souhaitons obtenir un portrait réaliste des facteurs contrôlant les flux d'énergie entre les compartiments microbiens.

Les avancements dans les méthodes –omiques sont parvenus à nous ouvrir les portes de la diversité microbienne. Maintenant que nous possédons une relativement bonne connaissance des espèces présentes, nous devons impérativement développer des méthodes nous permettant de quantifier l'impact biogéochimique de certains groupes-clés microbiens. Pour ce faire, nous devons conjuguer leur identification et leur dénombrement à des mesures réelles de leur taux métabolique. Ce n'est qu'une fois que cette association sera faite que nous réussirons à lier solidement la structure des communautés à la fonction de l'écosystème. Pour ce faire, il est essentiel de développer des méthodes de culture permettant de tester ces hypothèses *in vitro,* en s'assurant toutefois de le faire dans des milieux représentatifs des conditions *in situ*, notamment en incluant des communautés microbiennes complexes. De plus, à la manière des méthodes –omiques, un des plus grands défis qui se dresse devant les futurs océanographes microbiologiques sera de créer des méthodes de mesures à haut débit des taux métaboliques microbiens *in situ*. Il reste à voir si ces développements permettront de se libérer des traditionnelles incubations. Le développement continu de la cytométrie en flux, qui permet un dénombrement rapide des communautés microbiennes et l'utilisation de sondes et traceurs

variés (Hartmann *et al.,* 2009, Zubkov *et al.,* 2007), semble une avenue prometteuse (Wang *et al.,* 2010).

Par cette thèse, nous avons montré que le métabolisme microbien de l'océan arctique était une composante dynamique de l'écosystème marin. Nous montrons un rôle sous-estimé des communautés microbiennes dans les flux de C écosystémiques, menant à une révision de notre conception de la consommation du C par les bactéries. Cependant, toute une dimension de transformations pourrait potentiellement échapper au regard averti des scientifiques, en raison de la rapidité d'adaptation des voies métaboliques microbiennes (Ottesen *et al.,* 2013) à d'infimes changements de leur environnement (Stocker 2012). Dans le contexte du réchauffement climatique rapide de l'Arctique, il est primordial d'identifier le juste compromis d'échelle à considérer pour la conception de modèles plus réalistes. Pour ce faire, nous nous devons, dans un premier temps, de parfaire notre compréhension des processus microbiens locaux et de courte de durée. En effet, ceux-ci impliquent des changements très subtils dans la matière ambiante et sont présentement difficilement détectables par les méthodes de mesures classiques. Le développement d'outils d'échantillonnage autonomes sera instrumental à l'atteinte de cet objectif, en permettant une plus une haute fréquence d'acquisition de données à moindre coût. Ce n'est que lorsque nous aurons bien défini le rôle de ces microprocessus métaboliques que nous pourrons juger de la pertinence de leur inclusion – ou non – dans les modèles biogéochimiques globaux.

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